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EFFECTS OF IN UTERO IRRADIATION UPON POSTNATAL DEVELOPMENT IN THE MOUSE

by

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INTRODUCTION

Ionizing radiations are perhaps the most effective and consistent agent in teratogenesis, producing the most uniform effects on embryos within a given litter. This agent is particularly useful since its action has a general distribution throughout the organism so that patterns of sensitivity which are intrinsic to the embryo may be indicated by the selective response of structures. It is possible to time accurately both the onset and the duration of the treatment without having to determine such factors as the circulation and permeability of the agent within the maternal organism. Ionizing radiations also have the additional advantage of reaching the embryo almost immediately, making quantitative comparisons of different levels of irradiation more meaningful than with other agents of which it is difficult to determine exactly when they become effective since they may have to exceed a certain minimum amount before affecting the embryo.

The effects produced by ionizing radiations are peculiar to the embryo in so much as the same congenital anomalies cannot be produced by any amount of irradiation after birth. The embryo is unique in the course of its development because of the great number of cells that are actively undergoing differentiation. It is these cells that are most readily destroyed and altered by radiation. Studies in vertebrate radiobiology have clearly shown that the type of malformation

produced in the developing embryo is determined chiefly by the age of the embryo at the time of exposure, while the extent or severity of the malformation is influenced by the intensity of the radiation.

Most of the recent, more carefully controlled experiments in this field have been concerned with mainly the immediate (measured either before or just after parturition) effects produced by *in utero* irradiation with little study having been devoted to the long term results of embryonic or fetal irradiation. However, since an individual's development proceeds from conception until death, it was felt desirable to further systematically investigate the effects of *in utero* irradiation upon postnatal development. This study has consequently emphasized effects on development after parturition, although observations on prenatal effects as measured at term have been made. Response to *in utero* irradiation has been measured by gross, external morphological characters, growth from birth to 75 days of age, lifetime fecundity, and total lifespan.

It was also felt desirable to utilize in this study mice of several genetic backgrounds in order to obtain information on important genotypic-environmental interactions. Previous investigations have for the most part used either animals of unknown heterogeneous origin or else animals all of uniform origin. The use of several different genotypes makes it possible to make more valid generalizations from a single

experiment to an overall population of animals than if the results were gained from a single genetic background. The effects of *in utero* irradiation upon postnatal development in this study have been measured in three inbred strains of mice and all their possible hybrids.

REVIEW OF LITERATURE

Although there exists a large amount of literature on the effects of X-irradiation upon the mammalian embryo, much of the earlier work is difficult to evaluate, and the results often cannot be reproduced. Some of the early workers often did not appreciate the physical factors involved in irradiation with the consequence that there was little attempt to standardize these factors in experiments. Biological factors were also overlooked, some of the workers doing little in the way of determining the embryological stage at which irradiation was given, so that it is difficult to interpret any broad developmental patterns. Levine (1927), in fact, timed his embryos from the time the male and female were put together so that it is possible conception actually took place after irradiation.

In addition to the lack of adequate control of physical and biological factors, interpretation of the earlier studies is made further difficult because observations were on a relatively small number of animals, and the observations were limited almost exclusively to abortions and stillbirths. As a result many possible morphological abnormalities may have been overlooked. Russell (1954), for example, noticed in the photographs of Kaven (1938) certain abnormalities such as torsion of limbs and digital abnormalities which were not reported by Kaven.

In reviewing the effects of irradiation upon the embryo, the gestation period can be divided into several convenient points of division. Using the criteria of prenatal mortality and abnormality at birth, Russell (1950) found that the prenatal development of the mouse was divisible into three broad phases. The effects of irradiation were dependent upon the embryological stage at which the embryo was irradiated. These three divisions of prenatal development were in the mouse:

1) The preimplantation period (1/2 to 4-1/2 days). Irradiation during this period gave a high incidence of prenatal death but virtually no abnormalities among those embryos surviving to term. Russell did find 2 per cent abnormal embryos but these abnormalities were not in external characters.

2) The period of major organogenesis (5-1/2 - 13-1/2 days). Irradiation during this period caused virtually no prenatal loss of embryos but did cause a high incidence of different abnormalities at birth.

3) The period of the fetus (14-1/2 days to birth). Irradiation during this period of growth and minor organogenesis was not effective in causing prenatal death and did not cause any gross abnormalities at birth, although Russell (1950) and others have observed several types of abnormalities that do occur later in life.

These three divisions should only be considered as

convenient points for discussion and do not represent clear cut separations in the biological system, although they do represent general response patterns. Rugh (1959a) has shown that a certain anomaly, cerebral hernia or exencephaly, can be produced not only during early neurogenesis, but also at any time prior to neurogenesis, even at a time before the first cleavage and within hours of conception.

This result might possibly have been anticipated since the nervous system is closely dependent on other embryological morphological systems during its morphogenesis. In amphibian embryos, for instance, the initial differentiation of the nervous system is completely dependent on the underlying notochord-mesoderm tissue. It therefore seems reasonable to expect that even minor variations in embryonic regions during development could affect the highly sensitive nervous system and cause an abnormality in the central nervous system even before the latter was undergoing its neurogenesis.

It is possible, therefore, that Russell (1950) did not find any cases of exencephaly during the preimplantation period in her material either because of observing only a small number of animals or possibly because of intra-specific differences. Rugh found only a 2 per cent incidence of exencephaly, and Russell may simply not have observed it in her sample of 56 animals.

The literature has been reviewed here by placing each

work within one of these broad divisions. Some of the studies involve more than one period and are included therefore in each period. The emphasis has been placed on those works in which the physical and biological factors have been controlled relatively well. Reference is made, however, to some of the earlier experiments as they may be helpful when compared with some of the later, more carefully controlled experiments. This survey of literature has been greatly facilitated by a number of good reviews, the best of which are: Russell (1950), Russell (1954), Rugh (1953), O'Brien (1956), and Rugh (1959b). Russell (1954) is especially useful for a survey of the early literature in the field. Most of the papers reported in this review deal with experimental animals, and the clinical reports on effects of radiation on humans have for the most part been omitted. The reader is referred to other papers, for example, Murphy (1929), Russell (1954), Rugh (1959b), and Plummer (1952) for reports on the effects of radiation on human embryos.

An attempt at a comprehensive review of the literature of the experiments utilizing X-irradiation has been made. Only some of the experiments using other sources of radiation have been included.

Many of the works included in this review were concerned mainly with effects observed prenatally or shortly after parturition, but it was felt that these would be helpful in understanding some of the later, postnatal effects of <u>in utero</u>

irradiation. Most of the recent experiments have used either the mouse or the rat as experimental animals although guinea pigs and rabbits were also used during the earlier period of experimentation. In order to make inter-specific comparisons of the effects of in utero irradiation it is necessary to equate developmental and chronological development within each species. Using the three broad divisions of gestation given earlier, the corresponding chronological stages in each of these four species is as follows, preimplantation period: mouse - 0 to 5 days, rat - 0 to 7 days, guinea pig - 0 to 8 days, rabbit - 0 to 5 days; period of major organogenesis: mouse - 6 to 13 days, rat - 8 to 15 days, guinea pig - 9 to 25 days, rabbit - 6 to 15 days; period of the fetus: mouse - 14 to 19 or 20 days, rat - 16 to 21 or 22 days, guinea pig - 26 to 63 days, rabbit - 16 to 31 or 32 days. For a discussion on equivalent ages in mouse and human embryos the reader is referred to Otis and Brent (1952).

The procedure to be used in this review will be to present a summary of the results of experiments dealing with in utero irradiation for each of the three broad periods of gestation. In addition certain of the papers will be reviewed in somewhat greater detail.

Effects of *In Utero* Irradiation During the Preimplantation Period

A summary of literature on the effects of irradiation during the preimplantation period is given in Table 1. The measurement of the quantity of irradiation in each of the experiments has been left in the terms given by the original authors. In addition to roentgen and rad measurements, other systems of measurement include the Standard Erythema Dose (abbreviated SED), the Holzknecht unit (abbreviated H), and the pastille tint method.

The most significant point in these studies is that all of the workers reported prenatal mortality of embryos and with few exceptions reported the absence of abnormalities. Surviving mice in a litter appeared to be normal not only at birth but also during postnatal development (Kosaka, 1928e). The reported cases of arrested development are probably examples of dead embryos having been partially resorbed. Two important exceptions to the observations that surviving embryos are normal are the papers by Russell (1956) and Rugh and Grupp (1959). Whereas Russell found no abnormalities in external and gross visceral examination of newborn mice irradiated during the preimplantation period, she did find a low incidence of abnormalities in the skeleton of the vertebral column and thorax. The percentage incidence for any one abnormality at any single age did not exceed 6 per cent.

Table 1. Summary of experiments of irradiation given during the preimplantation period

Author	Dose[1]	Stage Observed[2]
	Species - Mouse	
Burckhard (1905)	30 min/exposure	0-8 PC
Parkes (1927)	40 min=1/4 B tint	6-10 PC, T
Russell (1950)	100r, 200r	12-17 PC, T
(1956)	100r, 200r	T
Russell and Russell (1956a)	200r	10-1/2 or 13-1/2 PC
Rugh and Grupp (1959)	50r, 200r	17-1/2 PC
	Species - Rat	
Bagg (1922)	? radium injection	? PC
de Nobele and Lams (1925)	1/2 - 2 SED	9, 14 or 21 PC
Job, et al. (1935)	0.8 skin unit	T ?
	12-90r	? PC, T
Hicks (1953)	300r, 400r	2 hrs PI to PN
Garmashev and Svetlov (1957)	200r-700r	?
	Species - Guinea Pig	
Trillmich (1910)	Wk. brown or tint B	PI to T (externally)

[1]Total body X-rays unless otherwise specified.

[2]Number of days postconception (PC), postirradiation (PI), term (T), postnatal (PN)

Table 1. (Continued)

Author	Dose[1]	Stage Observed[2]
	Species - Guinea Pig	
Kosaka (1928c)	1/6, 1/3, 2/3, SED	1 PI to T
	Species - Rabbit	
Saretzky (1908)	? Ovaries	?
Driessen (1924)	30H, 10H left side	14 PC
Momigliano (1934)	1/2-2 SED	?

These same abnormalities had a far greater incidence during postimplantation stages.

Rugh and Grupp (1959) reported in their material that none of 630 control implants showed any cerebral anomaly while in the irradiated group 2 per cent developed exencephaly. Exposures of less than 50r did not produce exencephaly.

It is evident in spite of these two exceptions that the early mammalian embryo is extremely radioresistant providing it survives at all. The effects are for the most part all-or-none effects, the embryo either dying in utero or else developing into a normal animal.

The question of the embryological stage at which the embryos die has been considered by several workers. Russell

(1950) reported that irradiation with 200r during the prenatal period increased prenatal mortality in the early stages. This dose given during gestation days 1/2 - 4-1/2 appeared to cause a significant reduction in the average size of litters brought to term. A dose of 300r given at 5-1/2 days was apparently 100 per cent lethal to embryos, with the death of the embryos appearing to occur early.

Rugh and Grupp (1959) reported that 50r given on any day before 9-1/2 days caused an average of 12.3 per cent of the embryos to die *in utero* compared to 5.7 per cent in the normal, unirradiated embryos. Of the unirradiated embryos that died *in utero* and were resorbed less than 0.3 per cent developed to fetal stages before death occurred. Even with 5r given at 1/2 day there was still 15 per cent uterine death. The apparent killing power of just 5r indicates that the early mammalian embryo is extremely radiosensitive.

Several other workers including Burckhard (1905), Parkes (1927), de Nobele and Lams (1925) and Driessen (1924) also examined the uterus within two weeks after pregnancy and found no signs of pregnancy in some of the females. These results would indicate that embryonic death had taken place before implantation providing, of course, that fertilization had actually taken place. Kosaka (1928c) found disturbances in implantation of three guinea pigs that were opened within 72 hours after irradiation.

Russell and Russell (1950a) in order to determine the sensitivities of different days within the preimplantation period examined uteri 10-1/2 or 13-1/2 days after mating. In general the earliest stages were the most sensitive. Between days 1/2 and 2-1/2 the average number of living embryos was only about 20 per cent of the controls. On day 3-1/2 it rose to 31 per cent and on day 4-1/2 to 57 per cent. It was observed that the deaths occurred quite early since at the time of observation at 10-1/2 days enough resorption had already taken place so that no abnormalities were recognizable.

In addition to loss of some members within a litter as a result of irradiation during the preimplantation period, there was a further increase in the loss of entire litters. Although all matings do not result in successful pregnancies, as evidenced by only 79 per cent of the control matings resulting in observable implantations, the number of unsuccessful matings in animals irradiated during the preimplantation period was only 56 per cent. They found that most of the embryos dying before implantation occurred in the groups that had been irradiated on days 1/2 and 1-1/2. Rugh and Grupp (1959) reported similar results in their material in which they observed that the most sensitive period with regard to uterine death and resorption was before the first cleavage or 1/2 day after conception at which time irradiation with 50r killed 42 per cent of the embryos and irradiation with 200r 64 per cent

of the embryos.

There is some question at present whether the normal appearing survivors of embryos irradiated during preimplantation stages are truly normal. Rugh (1959b) has emphasized the point that if the effects of irradiation are judged solely on the basis of a gross analysis, the results may be seriously in error. It is possible the central nervous system may exhibit certain neurological effects on a functional level which may not yield any detectable histological changes.

Effects of *In Utero* Irradiation During the Period of Major Organogenesis

A list of the experiments of irradiation during the period of major organogenesis is given in Table 2.

In marked contrast to the preimplantation period when *in utero* irradiation resulted in virtually no morphological abnormalities in surviving embryos, all recent workers have observed numerous abnormalities as a result of irradiation during the period of major organogenesis. Some of the earlier workers did not report any morphological changes, but some of the physical and biological factors had not been carefully controlled in these experiments.

Irradiation during the period of major organogenesis causes considerably less prenatal death than does irradiation during the preimplantation period. Russell (1950) observed that 44 per cent of female mice irradiated with 200r on days

Table 2. Summary of experiments of irradiation given during the period of major organogenesis

Author	Dose[1]	Stage Observed[2]
	Species - Mouse	
Parkes (1927)	10 min	T, PN
Kosaka (1927, 1928c)	1/8 - 2-1/2, total or parts	1/2 - ? PI, PN
Kaven (1938a)	200r abdomen	T, PN
Kaven (1938b)	200r abdomen	13-19 PC, T
Raynaud and Frilley (1943-1949)	5,000-200,000r head of embryo	18-1/2 PC
Russell (1950, 1956)	25-400r	T
Murakami (1952)	100, 150r	13 PC
Rugh and Wolff (1955)	300r	4, 24 and 72 hrs PI
Auerbach (1955, 1956)	300r or 100r(3x)	3-6 PI
Rugh (1956a)	50-300r	PN
Russell (1957)	25, 50, 100r	T
Russell and Major (1957)	100, 150r	PN
Carter (1958)	300r	PN
Murakami and Kameyama (1958)	25, 50r	13 PC
Fraser and Hall (1958, 1959)	250, 300, 350r	T, PN

[1]Total body X-rays unless otherwise specified.

[2]Number of days postconception (PC), postirradiation (PI), term (T), postnatal (PN).

Table 2. (Continued)

Author	Dose[1]	Stage Observed[2]
Grayevsky, et al. (1959)	400 and 600r	17 or 18 PC
Rugh and Grupp (1959)	25, 50, ..., 300r	17-1/2 PC
	Species - Rat	
Bagg (1922)	? radium injection	? PC
Hanson (1923)	? X-rays	T, PN
de Nobele and Lams (1925, 1927)	1/2, 2 SED	12, 14, 23 PC
Kosaka (1928b)	1/6 - 1-1/2 SED	1/4-10 PI, T
Job, et al. (1935)	12-200r	9-18 PC, T
Warkany and Schraffenberger (1947)	190-1120r lumbosacral	T
Hicks (1950, 1952, 1953, 1954a, 1954b, 1957)	35-600r	2 hrs PI - PN
Wilson and Karr (1951)	50-400r individual embryos	11-15 PC, T, PN
Wilson, et al. (1951, 1952, 1953a, 1953b)	125-400r	1 to several days PI
Levinson (1952)	300-600r	T, PN
Wilson (1954)	50-600r	1 to several days PI
Brent (1957)	400r (embryos shielded)	12 PI
Garmashev and Svetlov (1957)	200r - 700r	?

Table 2. (Continued)

Author	Dose[1]	Stage Observed[2]
Ershoff and Bavetta (1958)	150r	T, PN
Graham, et al. (1959)	150r, 300r	PN
	Species - Guinea Pig	
Trillmich (1910)	60 min	PI (externally)
de Nobele and Lams (1925, 1927)	1/2, 1 SED	20-55 PC, T
Dyroff (1927)	420r	T, PN
Kosaka (1928c)	1/6 - 1-1/2 SED	1/4 PI to T
	Species - Rabbit	
Sébileau (1906)	Tint no. 6	T, PN
Cohn (1907)	3 hrs (head)	PN
Fellner and Neumann (1907)	5-8 H upper 2/3 of abdomen	? PC, T
von Hippel and Pagenstecher (1907)	21 H	T
Saretzky (1908)	? Ovaries	PI
Pagenstecher (1916)	2-3.4 SED abdomen	28 PC
Nürnberger (1920)	15, 30 min abdomen	T, PN
Schinz (1923)	0.85 - 2 SED	PI to T
Driessen (1924)	10-78 H left side	10-26 PC
Kosaka (1928a, d)	1/6 - 2 SED	1/4 PI to T
Momigliano (1934)	1/2 - 2 SED	? PC

1/2 - 4-1/2 had no litters, compared to 31 per cent of females irradiated on days 5-1/2 - 8-1/2 and 28 per cent of control females. At still later stages, 9-1/2 - 13-1/2 days, when pregnancy was diagnosable, only 6 per cent of the females treated with 200r did not bear litters. These results indicate that susceptibility to loss of entire litters decreases fairly rapidly with embryonic age. A dose of 300r at 5-1/2 days apparently caused 100 per cent loss of entire litters.

Fraser and Hall (1958) in their material found that in the dose range of 250-350r irradiation between 8 and 14 days caused a greater number of females to produce no progeny at term than would be expected from a simple effect on litter size. The maximum effect occurred at 9 days when there were 59 per cent of the females that did not produce litters. An inspection of their figures indicates that all of the control females had litters. If their controls were handled in the same way as Russell's (1950), that is using only the vaginal plug as the criterion of pregnancy, then there would appear to be considerable difference in the two stocks of mice since only 72 per cent of Russell's control mice had litters.

Carter (1958) also mentioned that 36 per cent of his mice irradiated on day 13-1/2 did not yield litters. He does not, however, cite control figures.

In addition to the decrease in the loss of entire litters, there is also a decrease in the loss of individuals within a

litter as a result of irradiation during the period of major organogenesis. Kosaka (1927) found that a dose of greater than 1/2 SED would cause a 100 per cent prenatal loss between days 4 and 10, but a dose of greater than one SED had to be given to cause a 100 per cent prenatal loss after day 10. Kaven (1938b) also found the earlier stages in this period to be the more sensitive, a dose of 178r given on days 7 or 8 producing litter sizes of 4.3 and 4.7 respectively compared to the control mean litter size of 6.8.

Russell (1950) observed that a dose of 200r given between days 5-1/2 and 12-1/2 caused a decrease of only 11 per cent in the mean litter size at birth. The same dose given early in the preimplantation period, however, had caused an 80 per cent decrease. Most of the decrease in the 5-1/2 - 12-1/2 day period was due primarily to the reduced litter sizes shortly after implantation, 6-1/2 to 8-1/2 days. Murakami and Kameyama (1958) also observed an increase in susceptibility to resorption during the early postimplantation stages. Doses of 100r and 150r given on 8 days caused 20.9 per cent and 31.2 per cent resorption compared to 9.6 per cent in the controls.

Fraser and Hall (1958) reported a decrease in litter size throughout the period of major organogenesis as a result of irradiation with 250 - 350r. The greatest decrease occurred at 9 days. In their data the mean litter sizes were calculated by including females that had no progeny at all. If these

females are not included the mean litter sizes for 250r are not significantly different from that of the controls. The doses of 300r and 350r still appear to lower litter sizes significantly, the decreases at 9 days being 49 per cent and 46 per cent respectively. These authors state that the difference between their results and the results of Russell may be due to a higher rate of resorption of dead fetuses in their stock, although with doses of 300r or more Russell, with only a few litters represented, found litter sizes considerably reduced from irradiation between days 6-1/2 and 9-1/2.

The results of investigations using the rat are somewhat contradictory not only when compared to those of the mouse, but also when compared among themselves. Job, et al. (1935) using doses between 95-200r reported 100 per cent resorption. Warkany and Schraffenberger (1947) using considerably higher doses, 190-1120r, found that over the entire period of 9-15 days, 75 per cent of the females had litters. Wilson and Karr (1951) reported a dose of 200r given on the 10th day caused an 88 per cent reduction in litter size. The same dose given during a comparable stage in the mouse (8 days) caused only a 29 per cent reduction. Ershoff and Bavetta (1958) and Graham, et al. (1959) found no difference in respect to the number of litters cast, or the average litter size as a result of irradiation with 150r on 10 and 14 days.

In spite of these intraspecific and interspecific

differences in resorption rates in both species there was more prenatal death from irradiation of earlier stages in the period of major organogenesis. Kosaka (1928b) determined that in the rat only 1/3 SED was necessary to kill all embryos irradiated between days 5 and 10, but 1 SED was necessary between days 11 and 15.

The number of animals born dead as a result of irradiation during the period of major organogenesis is more frequent than for corresponding doses given during the preimplantation period. Neonatal death is markedly dependent on the stage irradiated. Kaven (1938a) found over 3/4 of the young were stillborn after irradiation with 178r on days 10, 11 or 12, but a smaller proportion was stillborn when irradiated just before or after these days.

Russell (1954) reported that doses of less than 100r had no effect on survival at birth when given on days 7-1/2 to 12-1/2. A sharp increase in neonatal deaths was produced by 200r when given at 9-1/2 and 10-1/2 days, the proportion being 75 per cent and 67 per cent respectively. A dose of 300r increased neonatal mortality in the same manner, reaching 100 per cent on days 9-1/2 and 10-1/2. The effect on neonatal mortality decreased sharply before and after these peak days. Russell estimated the LD_{50} at birth, or that dose which caused 50 per cent of the embryos to be stillborn, according to embryonic stage irradiated as follows:

Irradiation on days 1/2 - 8-1/2, $LD_{50} > 200r$

Irradiation on days 9-1/2 and 10-1/2, $LD_{50} < 200r$

Irradiation on day 11-1/2, $200r < LD_{50} < 300r$

Irradiation on days 12-1/2 - 15-1/2, $LD_{50} > 300r$

Investigations on the LD_{50} of rats at birth are incomplete, but Levinson (1952) reported that 50 per cent of the young were born dead after a dose of 600r at 13 days.

Several workers have reported decreased postnatal survival as a result of irradiation during the period of major organogenesis. Rugh and Wolff (1955) found that 300r given on day 13-1/2 caused almost all mice to die before the end of the first day, but the same dose given one day later enabled most mice to survive for at least six weeks. Carter (1958) found that 300r on 13 days in his stock caused only 54 per cent of the young to die in the nest.

Russell and Major (1957) reported on the effects of 100r and 150r given in the middle of the period of major organogenesis, namely 10-1/4 days, and found that whereas 92 per cent of the mice given 100r survived to 32 to 58 days, only 60 per cent survived after 150r.

Ershoff and Bavetta (1958) and Graham, et al. (1959) observed that mortality during the lactation period was significantly lower for rats irradiated with 150r at 10 days but not lower when irradiated at 14 days.

Effects on growth

There have been few studies on the effects of *in utero* irradiation during the period of major organogenesis on prenatal and postnatal growth. Cohn (1907) and Hanson (1923) mentioned reduced body size in adult stages in the rabbit and rat respectively. Kosaka (1928b, 1928c, 1928d) reported arrested development in several rodents from prenatally lethal doses.

Russell (1950) in an extensive experiment found that the mean birth weights of mice that had been irradiated between 8-1/2 and 13-1/2 days were considerably lower than those of the controls. The most critical period for both 200r and 300r appeared to be between the 10-1/2 and 11-1/2 day stages. The mean weight at 11-1/2 days for the 200r dose was only two-thirds of that of the controls.

Russell, Russell and Major (1951) extended the work on the 11-1/2 day stage and found that points for different doses and for the control fell on an approximately straight line, with weight reduction per 100r averaging 0.22 grams over the three available intervals.

Grayevsky (1959) mentions that irradiation of mice on both 6 and 9 days causes a "drastic reduction in their weight". It is evident from his graph that the highest dose, 300r, caused the mean weight to be only two-thirds that of the controls.

Raynaud and Frilley (1943-1949) utilized very local irradiation with a narrow beam in an attempt to selectively destroy the pituitary. Mice that were irradiated on days 12-1/4 and 13-1/4, with doses reaching a total of 200,000r, weighed only about 60 per cent as much as control litter mates.

Wilson and Karr (1951) found that although 50r given on day 10 had no effect on body weight 2-11 days after irradiation, a dose of 100r caused growth reduction of 37 per cent one day after irradiation. In the succeeding period up to term the reduction decreased in surviving embryos to between 6 and 15 per cent. A dose of 200r caused surviving embryos to be 20 per cent less than controls at birth. Seven of the animals that had received 100r weighing on the average 10 per cent less than the controls at birth, were raised and recovered this initial weight deficiency by 50 or 60 days postpartum. (Wilson, Jordan and Brent, 1953).

Hicks (1953) found a degree of runting common, but not constant in rats irradiated at any stage between the 9th day to term with 100r to 400r. He does not, however, give the actual reduction nor the day of irradiation.

Ershoff and Bavetta (1958) reported different results from the above from irradiation with 150r on days 10 or 14. They found no difference in the average weight of young at birth or at 21 days. Graham, et al. (1959) using the same treatments and the same strain of rats did find a 10 per cent

weight decrease at birth. Surviving rats, however, were "normal" at weaning.

Effects on morphology

Numerous morphological abnormalities have been reported as a result of irradiation during the period of major organogenesis. Most of the earlier works are difficult to interpret since careful control of the embryonic age at irradiation was not made. Von Hippel and Pagenstecher (1907) observed cataracts, microphthalmia and coloboma in newborn rabbits after irradiation with a dose of 21H on days 7, 9 and 11 or 8,10 and 12.

Kosaka (1927, 1928), although he did not determine the exact day of treatment, did irradiate during known intervals. He observed embryos 6 hours and more after treatment with a dose of 1/8 - 2 SED, and concentrated on histological descriptions of tissue damage. He found that the degree of sensitivity was dependent on the relative growth rate of the particular organs. The brain and spinal cord were most sensitive early in this period, followed in sensitivity by the retina. Certain organs did not show any marked effects at any stage from irradiation.

Job, et al. (1935) were the first workers to clearly demonstrate the presence of well-defined critical periods for morphological abnormalities. They gave a dose of 90r or less between the 8th and 11th days of gestation in the rat and

found the following percentage incidence: hydrocephaly - 7 per cent, jaw abnormalities - 6 per cent, eye defects - 22 per cent. The critical periods for these defects appeared to be: hydrocephaly - 9th day, eye defects - 10th day, and jaw abnormalities - 11th day.

Kaven (1938a) demonstrated the existence of critical periods in the mouse following irradiation with 178r. Brain hernias resulted from irradiation on days 7 or 8, with the peak being on day 8. These defects became apparent only late in prenatal life, usually after day 16. Tail abnormalities were found after treatment between days 9 and 14 with the peak of sensitivity on day 11. Hydrocephaly occurred in 10 per cent of the embryos irradiated on day 12, and there were also a few cases after treatment on days 10 or 13. Kaven also reported skin defects in later life following irradiation on days 13 or 14.

Russell (1950, 1956) did a detailed study of the skeleton and gross visceral structures following irradiation with doses of 100 to 400r between days 5-1/2 and 13-1/2. She found over 100 kinds of abnormalities including microphthalmia, polydactyly, oligodactyly, limb deformities, coloboma, vaulted cranium, spina bifida, imperforate anus, tail abnormalities, hydronephros, and open eyelids. Two important generalizations derived from this work are:

1. The critical periods for the induction of almost all

abnormalities are short.

2. The period of sensitivity is lengthened by increasing the dose.

Russell (1957) extended her studies on skeletal malformations induced by irradiation on days 7-1/2 or 8-1/2. She found that even a dose of 25r gave detectable changes in the vertebral column.

Wilson and Karr (1951) concentrated their studies on day 10 in the rat. Most embryos were examined one to five days after irradiation. A dose of 50r was almost completely ineffective in producing abnormalities, but the incidence of abnormalities was marked after a dose of 100r. Eye defects were the most common, occurring in 75 per cent of the animals. The authors emphasize that certain defects may not be detectable until the organs involved have reached a stage of differentiation adequate for observation. Central nervous system abnormalities were restricted almost exclusively to the telencephalon. Other organs such as the brain, heart, lungs and liver showed localized retardation of growth in addition to, or instead of, malformations.

Wilson, Brent and Jordan (1951) found neoplasia or tumor-like growths common after irradiation on day 9. A dose of 25r was adequate to cause 20 per cent incidence of head tumors in all embryos. It is likely that these growths were not actually tumors, but represented rosettes as reported by

several other authors, since very few of these tumors were recognizable at term.

Most of the changes observed as a result of irradiation early during the period of major organogenesis involved the central nervous system. Murakami and Kamayama (1958) concentrated on day 8 in the mouse and found that a dose of 25 or 50r produced increased incidences of hydrocephaly, brain hernias, pseudencephaly, microphthalmus, anophthalmos and malformed tail.

Rugh and Grupp (1959) observed the incidence of exencephaly following irradiation with doses of from 25 to 300r to mouse embryos of 6-1/2 to 10 days. They found that 25r produced no cerebral hernias, but that 50r did produce 5 per cent on day 8. A dose of 200r extended the critical period from 6-1/2 to 9 days. The results showed that in general days 7-1/2 to 9 were the most sensitive. Even a dose of 300r produced no exencephaly on day 10 indicating that the embryo had evidently gone too far in its development by 10 days to react in this manner.

Rugh (1959b) emphasizes that the apparent normality of litter mates of embryos with exencephaly is probably not real. Since embryonic cells damaged by irradiation cannot be replaced, various kinds of deletion, including microphthalmia, microcephalia and anencephalia, may occur. However, as the developing embryo has certain regulatory powers that enable it

to integrate the remaining undifferentiated, undamaged cells, the organism may appear topographically normal, but is actually reduced or deficient in certain aspects. This view is supported further by earlier evidence by Rugh and Wolff (1955). They studied the responses of the eye of mouse embryos after irradiation at 12-1/2 or 13-1/2 days with doses from 50 to 300r. Their observations made at 4, 24 and 72 hours and 6 days following exposure revealed that the reparative power of the fetal eye was considerable. Severe damage of the presumptive retina was evident even four hours after irradiation. However, by 72 hours the retina appeared to be normal, and there was no gross evidence of any damage at birth, although the eyes of mice observed at two months were microphthalmic. A dose of 250r at 12-1/2 days reduced eye volumes to 51 per cent of the controls.

Hicks and his co-workers in a series of papers (1950-1957) reported on extensive studies of the nervous system following <u>in utero</u> irradiation. Necrosis of embryonic neuroblasts was evident within two hours of irradiation. Although phagocytosis within six hours helped to clean up the cellular debris from necrotic areas, rosettes were seen to develop in neural tissue. Even a dose of 40r would necrotize some neuroblasts. A summary of defects found in rat embryos according to stage irradiated is as follows (Hicks, 1953):

Embryonic Stage	Defects
Presomite, early neural plate, (late 9th day)	anencephaly, pseudencephaly
4 Somites	anophthalmia, microphthalmia
10-12 Somites	virtually complete recovery
20 Somites (11th day)	encephalocoele of ventricles recurrence of ocular defects, hydrocephalus, craniospinal abnormalities, visceral anomalies
30 Somites (12th day)	cerebral deformities
40 Somites (late 13th day)	syndactyly, forebrain defects
Early neonatal period (13th day)	abnormalities of the brain, microcephalies decreasing in gross severity through the 18th day with cerebellar anomalies beginning about the 14th day, occasional stunted growth.

Auerbach (1956) found that the incidence of spina bifida and coloboma was not affected by fractionation of X-rays, but that the severity was far greater in response to a fractionated dose. He exposed 9-1/2 day mouse embryos to a single dose of 300r or to three 100r fractions at intervals of 30 minutes. This is the only evidence so far that demonstrates

that short-interval fractionation can increase the radiation hazard to developing embryos *in vivo*.

Fraser and Hall (1958) reported that irradiation between days 8 and 14 either increased or decreased the number of facial vibrissal in the mouse depending on the stage irradiated. No effects were observed after a dose of 250r, but doses of 200 and 350r caused an increase in vibrissae number on days 9 and 10 and a decrease on days 11 to 13. They also found 12 cases of newly born mice with naked patches of skin. These cases occurred in some of the progeny of litters that had been irradiated on 10, 11 or 12 days with 350r.

Other effects

Several workers have reported postnatal changes in behavior as a result of *in utero* irradiation. Levinson (1952) found that the learning ability of rats was decreased, as a function of the doses given, when irradiation was between days 11 and 12 with doses from 300 to 600r. Embryos irradiated on day 13 were found to be more sensitive to irradiation than embryos treated earlier or later in gestation. Levinson observed that the irradiated mice also exhibited more nervousness in the maze as evidenced by teeth chattering, persistent scratching, face washing, defecation and urination.

Rugh (1956b) found that mice that had been irradiated at days 9-1/2 to 17-1/2 with doses from 50 to 300r exhibited more nervous excitability than controls when tested at 15 days and

two months after birth. At the earlier fetal ages, 12-1/2 to 13-1/2 days, even 100r caused detectable changes in behavior. The dose necessary to detect changes increased with embryonic age. It is probable that behavioral changes are as sensitive in detecting impairment in the central nervous system as are morphological changes.

Ershoff and Bavetta (1958) observed that the severity of dental caries, but apparently not the incidence, observed in rats 90 days of age, was greater in animals that had been irradiated as 10 day embryos with 150r. There also appeared to be some effect of irradiation at 14 days but not at 18 days.

Graham, et al. (1959) found that 150r at 14 days significantly impaired discrimination learning performance, but the same dose given on days 10 or 18 did not have this effect. A dose of 300r on day 18, however, also impaired learning performance.

Russell and Major (1957) irradiated mouse embryos, heterozygous for four coat color genes, with 100r or 150r at 10-1/4 days, and observed them as adults for mosaic patches. They found that the somatic mutation rate was of the same order of magnitude as the germinal mutation rate in spermatogonia. Irradiation had apparently caused some killing of prospective pigment cells resulting in mosaics.

Carter (1958) irradiated mouse embryos with 300r and

mated male offspring to a female tester stock carrying seven mutants. He found that the yield of induced mutation, as recovered from spermatozoa of the adult, is lower for foetal than adult spermatogonia.

Effects of Irradiation During the Period of the Fetus and the Neonatal Period

A summary of experiments of irradiation given during the period of the fetus or to newborn animals is given in Table 3. There have been few experiments concerned exclusively with the effects of irradiation during the period of the fetus and the neonatal period. The available evidence indicates that there is considerably less mortality than for equivalent doses given during the period of major organogenesis. This is evident even in some of the earlier experiments such as Burckhard (1905) and Trillmich (1910) who found no effect on mortality as a result of irradiation during the period of the fetus with doses that caused considerable prenatal mortality during the period of major organogenesis.

Spalding, et al. (1958) irradiated mice between the 14th and 18th day of gestation with a dose of 400 to 700r, and found that the ratio of live-to-dead births decreased with increasing dose. The percentage of animals born dead went from 22.1 per cent in the 400r group to 57.9 per cent in the 700r group. These authors did not state the exact day of exposure, but did give the number of days after exposure that

Table 3. Summary of experiments of irradiation given during the period of the fetus and to newborn animals

Author	Dose[1]	Stage Observed[2]
	Species - Mouse	
Burckhard (1905)	?	T, PN
Nürnberger (1920)	5 min abdomen	T, PN
Kosaka (1927, 1928e)	1/8 - 2-1/2 SED total or parts	1/2-? PI, PN
Parkes (1927)	10-40 min	T, PN
Kaven (1938a)	200r (abdomen)	T, PN
Hicks (1950)	35-600r	2 hr PI-PN
Abrams (1951)	550r (N)	PN
Vogel (1951)	1,000r (head)(N)	PN
Rugh (1952)	50r (6X)	PN
Brunst and Figge (1953)	3,000-4,000r abdominal band (N)	PN
Levy, et al. (1953)	300r	T, PN
Grobman (1954)	300r, 500r	T, PN
Deringer and Lorenz (1955)	400r (N)	PN
Rugh (1956a)	50-300r	PN
Rugh and Wolff (1957)	10-300r	PN
Benedict (1958)	100-300r (N)	PN

[1]Total body X-rays unless otherwise specified. Animals irradiated post-parturition (N).

[2]Number of days postconception (PC), postirradiation (PI), term (T), postnatal (PN).

Table 3. (Continued)

Author	Dose[1]	Stage Observed[2]
Spalding, et al. (1958)	400-700r	T
Rugh and Jackson (1958)	50-200r	PN
	Species - Rat	
Bagg (1922)	? Radium	T, PN
Hanson (1923)	?	T, PN
Kosaka (1928b)	1/3 - SED	1/4 PI-PT
Job, et al. (1935)	0.2-1.6 skin unit 12-90r	T
Hicks (1950)	35-600r	2 hrs PI-PN
Stearner and Christian (1951)		PN (N)
Levinson (1952)	300-600r	T, PN
Schwarz, et al. (1952)	150-500r	24 hrs PI-T
Tait, et al. (1952)	30-360r	PN
Hicks (1953)	100-400r	T, PN
Shaver (1953)	300-500r (N)	12 hrs PI-PN
Ershoff and Bavetta (1958)	150r	T, PN
Furchtgott, et al. (1958a, 1958b, 1958c)	100-300r	T, PN
Graham, et al. (1959)	150, 300r	T, PN
	Species - Guinea Pig	
Lengfellner (1906)	20-60 min	T

Table 3. (Continued)

Author	Dose[1]	Stage Observed[2]
Trillmich (1910)	60 min	PI externally
Nürnberger (1920)	?	T, PN
de Nobele and Lams (1925, 1927)	1/2-1 SED	37 PC-PN
Dyroff (1927)	350r	T, PN
Kosaka (1928c)	1/3-2 SED	1/4 PI-T
	Species - Rabbit	
Sébileau (1906)	Tint no. 3	T, PN
Cohn (1907)	3 hrs (head)	PN
Fellner and Neumann (1907)	5-8H upper 2/3 of abdomen	T
Saretzky (1908)	? ovaries	PI
Nürnberger (1920)	30 min abdomen	T, PN
Lacassagne, et al. (1922, 1923)	5-1/2 - 13H	29 PC - 10 PN
Schinz (1923)	1/4 - 2 SED	PI-T
Kosaka (1928a)	1/3 - 2 SED	1/4 PI-T
Momigliano (1934)	1/2 - 2 SED	T

the litters were born. By working backward it is then possible to determine the apprcximate age at irradiation. It can be seen that most of the increase in stillborn embryos

occurred as a result of irradiation during the earlier part of this period on days 13-1/2 to 15-1/2.

Graham, et al. (1959) irradiated rats on day 18 with 150r or 300r and observed that mortality during the period of lactation appeared to be greater than that in the control. The percentage mortality was 0r - 7.0 per cent, 150r - 9.8 per cent, and 320r - 13.0 per cent. The authors did not cite the actual number of mice involved so that it is not possible to determine if these differences are significant.

Zuikova, et al. (1959) reported in an abstract that in dogs irradiated during the last trimester of pregnancy, the duration of life was in direct relation to the dose. They found profound morphological changes in the tissues and membranes of the brain and in the urinary bladder. They attributed much of the poor viability and underdevelopment of the young to congenital atelectasis of the lungs.

The effect of irradiation on survival decreases rapidly with increasing embryonic age and increasing age after birth. Abrams (1951) found that a dose of 550r killed 22.5 per cent of day old mice by 30 days of age, and 60 per cent by 60 days of age.

Sterner and Christian (1951) irradiated rats with 400 to 1,000r at 1/2 to 5 hours, 1 day and 2 days after birth. They observed that radioresistance increased rapidly after birth and at 2 days was only slightly less than that of adults. In

this study as well as the previously mentioned ones, most of the mortality reported has occurred in the two week period following birth, the gross changes being typical of radiation sickness, as evidenced by anemia, hemorrhages in the viscera and subcutaneous tissues, and atrophy of the spleen, thymus, and lymph nodes. Sterner and Christian observed marked generalized edema, hyperemia and multiple petechial hemorrhages in the kidneys within 24 hours after treatment.

Rugh and Wolff (1957) found that an improvement of tolerance of X-irradiation to a dose of 525r at four months of age could be obtained by virtue of exposure to 10r while in utero between days 13-1/2 - 16-1/2. The maximum effect was obtained on day 15-1/2 when there was a 17.4 per cent improvement in the males and a 24.4 per cent improvement in the females. The mechanism of this event remains to be elucidated and is somewhat puzzling since higher exposures of 25r to 300r to any age fetus were shown to have a deleterious effect in later life. These authors have not actually tested the significance of their results. A chi-square analysis of these data by this reviewer shows that these differences in survival are not statistically significant, and do not actually show that a dose of 10r in utero causes an improvement in tolerance to X-irradiation.

Effects on morphology

Morphological changes as a result of irradiation during the period of the fetus and the neonatal period often do not become expressed until later in life. Several workers, however, have made observations of the effects of irradiation during the period of the fetus, within a short period after irradiation. Kosaka (1928b, 1928c) sectioned rat and guinea pig embryos from 6 hours to term after irradiation with 1/3 - 2 SED during the fetal period. The most tissue damage in the rat was found in the brain, retina and thymus. There was some damage in the liver and spleen and still less in the skin.

Hicks (1950) observed rat and mouse embryos 1/2 to 96 hours after irradiation in addition to various ages after birth. He found 100 per cent damage in the brain, and usually in the retina, cord and ganglia after doses of 200, 400 or 600r. Hicks concentrated his studies on the central nervous system and also found changes in the postnatal period, including virtual absence of corpus callosum, and jumbling and reduction of the hippocampus. Damage outside of neural tissue was rare. It has already been mentioned that neuroblasts are present throughout the period of the fetus and into the neonatal period. This accounts for the prolonged sensitivity of the nervous system.

Schwarz, et al. (1952) used infra-red spectroscopy to examine brain tissue after irradiation. Rats were irradiated

during the last week of pregnancy with doses of 150r to 400r, and most fetuses were examined 24 hours after exposure. They found that X-rays caused an increase of the amide-free lipid to protein ratio in fetuses, with the effect being more frequent at doses of 300r or greater. Histological studies revealed changes similar to those described by Hicks (1950). The destructive effects of X-rays upon germinal areas of the forebrain were reflected in the results of the chemical breakdown of embryonic cell elements.

Bagg (1922) was one of the first workers to report on delayed effects of irradiation. Rats which survived acute radiation sickness exhibited opaque pupil and atrophied lens, and at autopsy at about one year of age had small cerebral hemispheres, and small ovaries or testes. Kosaka (1928a) also noted marked hypoplasia of the cerebrum and the gonads in mice irradiated with 2/3 SED after day 14.

Levy, et al. (1953) irradiated mouse embryos of 15-1/2 days with 300r and examined the femur, mandible and parietal bones at birth and various other days to 240 days of age. Irradiated embryos were born with bones having dimensions smaller than normal. There were significant differences between averages on the various days for both control and irradiated animals for all measurements between one and 29 days after birth. In general, the irradiated animals maintain smaller bone dimensions compared with the unirradiated

animals, although the differences are not as marked as time goes on. These results are to be expected from embryology since by 15-1/2 days in the mouse, the primary centers of ossification are evident, and any radiation effects should therefore be on the process of growth and not on initial differentiation.

Abrams (1951) and Brunst and Figge (1953) irradiated newborn mice utilizing whole-body and partial-body irradiation respectively. Abrams found that mice surviving a dose of 550r exhibited marked stunting, physiological immaturity and poor neuromuscular coordination. Brunst and Figge used a narrow transverse band of irradiation given either to the head or to a region midway between fore and hind limbs. In the group irradiated in the head they observed suppression of head development and development of the body as a whole as early as one week after irradiation and noted brain paroxysms at 9 days. The second irradiated group exhibited paralysis, usually in those animals that had been irradiated exactly in the region of the motor neurons controlling the hind limbs.

Several workers have observed cataracts as a result of irradiation during the fetal period. Kaven (1938a) and Russell (1950) reported development of cataracts later in life, but did not report percentage incidence nor the critical period for the induction of cataracts.

Vogel (1951) irradiated 2 day old mice with doses up to

1,000r to the head, and removed eyes for observation 1 to 22 days after irradiation. He observed no cataracts in eyes of mice exposed to less than 400r. No definite lens opacities could be seen by direct visual examination 4 to 5 months after treatment. All mice that received 900r or more developed cataracts usually within 2 to 4 weeks. In the range of 400 to 800r not all eyes showed cataracts. Damage seemed to be progressive and given a long enough latent period the percentage of cataracts in these groups were expected to increase.

Benedict (1958) irradiated newborn mice with doses of 100, 200 or 300r and observed the eyes at intervals up to over a year. All mice that had received a dose of 200r or more had a lens opacity at the first examination at 2-1/2 weeks. Most of the cataracts induced with 200r remained constant or actually regressed in density as the mice grew older. Mice receiving a dose of 300r did not show regression of the nuclear opacity. No lamellar opacities were induced by 100r.

Effects on the reproductive system

Little systematic work has been done on the effects of irradiation during the period of the fetus on subsequent reproductive capacity of the embryo. Nürnberger (1920) reported sterility in the only guinea pig, a male, that survived the early postnatal period. Hanson (1923) noted that "nearly all" rats irradiated in fetal stages proved

sterile. Parkes (1927) tested mice that had been irradiated between days 10 and 18, mostly between days 14 and 17, and found only 4 of 16 females and neither of two males fertile. Kosaka (1928e) observed that irradiation on days 7 and 13 did not affect fertility, but that irradiation after day 14 completely sterilized all but two males. These males had been treated on day 17 and were only temporarily fertile. Sterility appeared to be due to the failure of sperm formation. Females were mostly poorly fertile, showing reduction in the number of mature ova formed.

Shaver (1953) studied the effects of irradiation with 300r on newborn or 2 day old rats. The rats continued to gain weight, but retardation was apparent when compared to littermate controls. Animals were sacrificed at ages from 3 days to 34 weeks. At three days after irradiation spermatogonial mitoses were arrested and many spermatogonia were necrotic. At subsequent periods there was a relative decrease in the number of germ cells. No regeneration occurred in the testes at intervals up to 34 weeks. Regeneration was observed in a small number of seminiferous tubules of animals irradiated between 3 and 5 days after birth.

Rugh (1952) gave mice 300r, fractionated into 6 doses of 50r a day for 6 days beginning on the 14th day of gestation. At 6 months he tested them for fertility and found that 2/3 of the offspring were sterile. There was slightly greater

sterility among males but a greater number of first generation teratologies from the females. All of the irradiated males showed some testicular damage.

Grobman (1954) examined 29 mice at 37 to 49 days of age following irradiation with 300r on day 14-1/2 to 15-1/2 of gestation and observed reduction in size of gonads and secondary sex glands, absence of corpus callosum, and absence of gall bladder. Defects were also observed after irradiation on days 11-1/2, 12-1/3 or 13-1/2.

Deringer and Lorenz (1955) irradiated newborn strain HR mice with 400r. They found ovarian tumors more frequent in irradiated animals and reduction of ovaries in some of the females. The principal effects on the males were reduction in size of the testes and destruction of some of the seminiferous tubules.

Rugh and Jackson (1958) did a carefully controlled experiment in which mouse embryos, between days 15-1/2 and 18-1/2 were exposed to doses from 50 to 200r. At 2 months of age treated mice were mated to unirradiated mice for a 6 month period. The results indicate that the fetal female can tolerate more irradiation than can adult females without appreciably affecting fertility. Even a dose of 50r appeared to have some effect. After day 16-1/2 the fetal ovary appeared to be quite radioresistant. Fetal male mice were even more drastically effected by <u>in utero</u> irradiation. The

effect on the gonads appeared to be permanent, remaining steady throughout the period of observation. These results, as well as those of other workers, indicate that the fetal testis is more radiosensitive than the fetal ovary, which is just the reverse of the situation found in the adult testis and ovary.

Effects on behavior

Tait, et al. (1952) was one of the first workers to study the effects in rats of in utero irradiation upon learning ability in later life. Unfortunately he did not critically time the age at irradiation, except that it was during the last week of gestation. He used doses from 30 to 360r, and found that the animals receiving 90r or more were significantly poorer maze learners.

Furchtgott, et al. (1958) extended the work of Tait, et al. (1952) and Levinson (1952) by using several doses and including irradiation of newborn rats. They also used other behavioral criteria in addition to learning. They gave doses from 100 to 300r to 14, 16, 18 and newborn rats. They found that the maximum learning deficit occurred at day 14 when even 100r was effective in decreasing maze learning. Sensitivity decreased with advancing age, a dose of 200r at 18 days and 300r to newborn rats then being necessary to lower learning ability. They also measured the ability of rats to traverse normally along two narrow parallel bars, with the distance between the

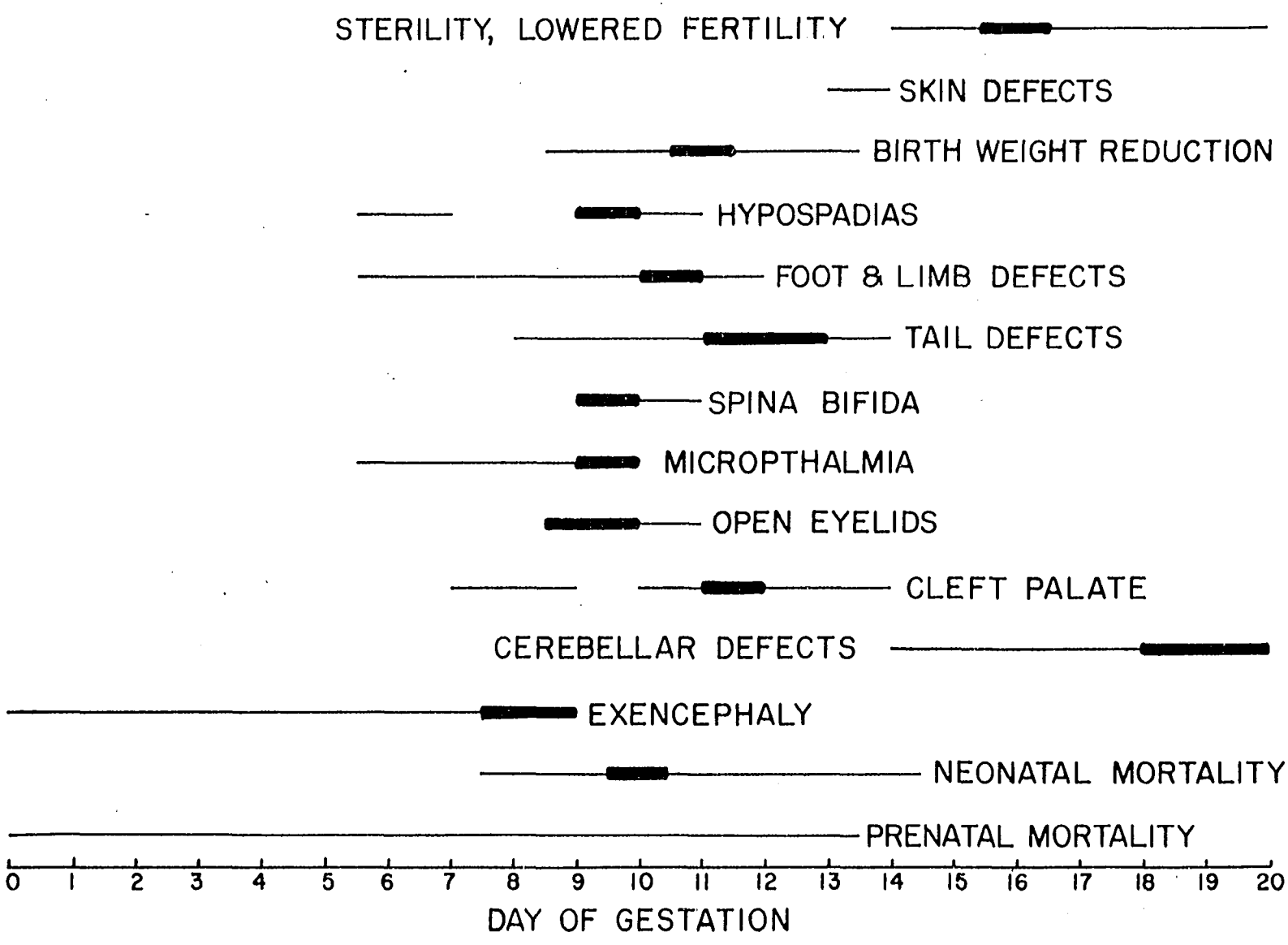
bars as the major independent variable. They observed that animals treated with 50 to 300r between day 14 and birth were inferior in locomotor ability to control animals when tested at 27 days. Coordination appeared to be more affected by radiation in the later part of gestation and neonatally than was maze learning. These results are in agreement with the morphological changes observed by Hicks, who found the cerebellum was maximally radiosensitive later in development than any other central nervous system structure. Additional tests were made utilizing tilting cages, open field behavior and home cage emergence. Their results in general showed that irradiation during the period of the fetus and the prenatal period produced hyperemotionality.

A general timetable of some of the main types of malformations induced by X-irradiation of the mammalian embryo is shown in Figure 1.

Intra-Specific Differences in Response to *In Utero* Irradiation

Although intra-specific differences in response to irradiation of adult mammals have been found for many kinds of response, there have been virtually no investigations to see if the same kind of differences exist in response to irradiation of the embryo. Russell and Russell (1950b, 1954) studied the effect of genetic constitution on radiosensitivity to the induction of homeotic shifts in vertebral borders and related

Figure 1. Timetable of some of the irradiation malformations observed in the mouse. The wide portion of each line indicates the period of peak incidence for that abnormality. The remainder of the line indicates that the abnormality is observed to some degree, more or less, following irradiation of the embryo on those days.


STERILITY, LOWERED FERTILITY
SKIN DEFECTS
BIRTH WEIGHT REDUCTION
HYPOSPADIAS
FOOT & LIMB DEFECTS
TAIL DEFECTS
SPINA BIFIDA
MICROPTHALMIA
OPEN EYELIDS
CLEFT PALATE
CEREBELLAR DEFECTS
EXENCEPHALY
NEONATAL MORTALITY
PRENATAL MORTALITY
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAY OF GESTATION

changes in the thorax. They used three populations of mice, consisting of two inbred strains (BALB/C and 129) and a hybrid (C57 X NB) population. They irradiated embryos between days 7-1/2 and 12-1/2 and found differences between the three strains. A dose of 200r on day 8-1/2 increased the number of presacral vertebral to 27 in 100 per cent of BALB/C mice, in only 3 per cent of the hybrid mice, and in 0 per cent of the 129 strain. However, the control results showed that the BALB/C and 129 distributions were situated across the 25/26 threshold, while the hybrid distribution crossed neither threshold. The authors concluded that the apparent differences in sensitivity were not necessarily the result of differences in sensitivity to primary radiation damage, but were probably due to differences in developmental potencies, and that it was the genetic constitution which determined the location of the strains on this scale of developmental potencies.

MATERIALS AND METHODS

Biological

The mice used in these experiments were taken from 3 inbred strains maintained at the Genetics Laboratory of Iowa State University as part of Dr. Gowen's Atomic Energy research program. These strains have been inbred many generations by brother-sister matings. The strains were originally differentiated by resistance to mouse typhoid caused by Salmonella typhimurium (see Gowen, 1948, for review). They have been used extensively in genetic experiments, and in addition to their differences in disease resistance, are known to differ in a number of other physiological characters including differences in response to various effects of irradiation (Grahn, 1954; Gowen and Stadler, 1956; and Stadler and Gowen, 1957). The strains used in this study as designated by the Committee on Mouse Nomenclature are the BALB/Gw (henceforth abbreviated as BaB), K and S.

Animals that were to be irradiated as embryos were obtained by mating virgin females, 2 to 3 months old, to males of approximately the same age. Matings were made between the 3 inbred strains, BaB, K and S, and between all of their possible hybrids including reciprocal matings. These matings produced progeny which were either inbred or hybrid, representing 9 genomes as presented below.

		Strain of male parent		
		BaB	K	S
		Genotypes of Progeny		
Strain of female parent	BaB	BaB/BaB	BaB/K	BaB/S
	K	K/BaB	K/K	K/S
	S	S/BaB	S/K	S/S

The diagonal of this rectangle represents embryos which are inbreds, having received both maternal and paternal sets of chromosomes from the same strain of parent. The off diagonal progeny represent embryos which are hybrids, having received maternal and paternal sets of chromosomes from different parental strains. There are two kinds of hybrids, depending on whether or not a particular set of chromosomes was contributed by the maternal or paternal strain.

Mated females were examined daily for the presence or absence of a vaginal plug. The male was removed from the cage the day after a plug was observed in the female. The appearance of the vaginal plug was the sole criterion used to time the period of gestation, the approximate age of the embryo being determined from this time. However, a number of factors are operative which make the estimate of developmental stage based on the time of conception subject to an uncertain amount of error. There is considerable variation not only between litters, but also within individuals of the same litter as to fertilization time, implantation time, and

subsequent developmental rate. Allen and MacDowell (1940) observed that embryos of the same chronological age may differ as much as 24 hours in developmental age.

Although the majority of matings in mice take place during the night, they have been known to occur throughout the entire 24 hour period. In this experiment, nevertheless, all mice were considered to have mated during the same time of night. The time of 4:00 a.m. was chosen as the approximate time of fertilization in all females since Snell, et al. (1940) determined that in the Bagg strain of mice the modal ovulation period was between midnight and 3:00 a.m., with fertilization occurring shortly after liberation of the egg. All pregnant females were irradiated at about 4:00 p.m. so that the embryological ages used in this study were approximately 6-1/2, 10-1/2, 14-1/2 and 17-1/2 days, although it is possible that each individual estimate may be actually a day more or less.

The in utero experiment was designed as a factorial with 3 genetically differentiated strains of mice and all their possible hybrids making a total of 9 different inheritance types, 4 levels of x-irradiation, and one treatment group at the 0r (control) level for each inheritance type. The 4 embryological ages, 4 levels of irradiation and one control group combined with the 9 different inheritance types yielded a total of 153 different experimental groups.

Pregnant females were examined at least twice daily, in the morning and in the early evening, so that newborn litters were usually found within 12 hours of birth. Mice in a litter were individually marked at birth by means of india ink injected by a hypodermic needle underneath the skin. Individuals were then given a gross, external morphological examination, and checked for the following characters: eyelids open or not; presence of cranial blisters; length and shape of the tail; overgrowth or reduction of the number of digits, plus any other outstanding morphological abnormalities.

The progeny were checked for deaths daily and individually weighed at birth, 12, 26, 40, 60 and 75 days of age on a (gram-atic) balance. Birth weights were recorded to the nearest hundredth of a gram, and all other weights to the nearest tenth of a gram. Between 7 days and 12 days of age mice were permanently marked by toe clipping. All mice were weaned at 30 days, and the males and females separated at that time.

At 75 days of age mice were individually mated to non-irradiated mice of the Z strain, a strain noted for the regularity, frequency and size of its litters. Virgin females and males 75 $\pm$ 15 days old were used in these matings. These matings are being continued for the lifetime of the irradiated mouse.

In order to have a balanced number of mice being tested

for reproductive capabilities in each of the treatment groups, it was decided to use 2 males and 2 females from each of the 153 treatment groups. Thirty-six of the 153 treatment groups did not provide any progeny that lived to 75 days of age. This resulted in a total of 468 mice being tested for breeding performance. At 75 days of age 2 males and 2 females were randomly chosen from each litter. If a litter did not contain 2 males and 2 females, whatever was available was used, and an additional litter from another female raised to 75 days to obtain the additional mice to fill a cell of 2 males and 2 females. Except for the testing of reproductive capabilities, the experiment had unequal subclass numbers due to differential litter sizes and differential postnatal viability.

When the Z mouse in a mating died, it was replaced within a few days by another mouse of the Z strain, between 2 and 6 months of age. Virgin females were used throughout the experiment as replacement mice. All matings were maintained for the lifetime of the treated mice even if they did not yield any progeny.

All matings between mice irradiated as embryos and mice of the Z strain were observed daily and the following information recorded:

1. Life span of the treated mouse
2. Total number of litters
3. Number of progeny within litters

4. Weight at birth of individuals in first litters

5. Viability of litters from birth to 21 days

6. External abnormalities of litters

A second experiment was undertaken in order to determine the effects of x-irradiation upon newborn mice. The procedures in this experiment were similar to those in the in utero experiment with the following exceptions. Newborn litters were irradiated at 4:00 p.m. on the day they were born. The dam did not receive any irradiation. The same 4 levels of irradiation were used, 20r, 80r, 160r and 320r plus one control group (0r). The 5 levels of irradiation combined with the same 9 inheritance groups represented in the in utero experiment resulted in a total of 45 treatment groups. With 2 males and 2 females per cell, a total of 180 mice were tested for breeding performance. Unequal subclass numbers are found in all parts of this experiment also except for the testing of reproductive capabilities.

It was necessary to use disproportionate frequency analyses of all the data because of the unequal numbers of mice. The statistical procedures used in the analysis of the data are essentially as those described by Snedecor (1956), and additional details will be explained with the presentation of results.

All mice used in this experiment were maintained in a well ventilated room, in which the environment and management

were relatively constant. Food and water were provided *ad libitum*.

Physical

The source of irradiation was a General Electric Maxitron which operated at 250 pkv, 30 ma with 0.25 mm Cu + 1 mm Al filtration at a distance of 50 cm from anode to mid mouse. The dose rate was approximately 133r/minute, the dosage rates having been measured in air by means of a rate meter. The exposure times for the various doses were; 20r : 9 seconds; 80r : 35 seconds; 160r : 1 minute, 12 seconds; 320r : 2 minutes, 25 seconds. The amount of radiation actually received within the body cavity of the female mouse was estimated by placing a rate meter inside a mouse from which the organs had been removed. It was found that approximately 90 per cent of the amount of radiation measured in air reaches the internal organs.

In the *in utero* experiment pregnant females were irradiated in a circular wooden container, 6-1/2 inches in diameter, 1 inch in depth. The base of the container was 1/4 by 1/4 inch wire mesh, and the top was covered with two layers of cellophane. Mice were further restricted within this container by an oval-shaped piece of cardboard which limited the animal to the immediate area around the opening of the container. Pregnant mice were exposed to single doses of *whole-body* irradiation. The number of mice irradiated at any

one time varied from one to three. After irradiation mice were immediately returned to their individual cages.

In the experiment in which litters were irradiated at term, the entire litter was exposed to whole-body irradiation in small, plastic trays, and then immediately returned to their dam.

EXPERIMENTAL RESULTS

Effects of In Utero Irradiation upon Morphology

It was expected from the work of previous investigators that the embryological age of 10-1/2 days would be the only stage when irradiation would produce external, morphological abnormalities observable at birth. The results confirmed these expectations since the only 3 treatments which yielded abnormalities in more than singular occurrence were with doses of 80, 160, or 320r at 10-1/2 days. It was also expected from the work of Russell and others what types of abnormalities might be found as a result of in utero irradiation. All abnormalities that were evident upon a gross, external examination were recorded. The main kinds of morphological changes and a possible explanation of the underlying anatomical causes as given by Russell (1950) are:

1. Eyelids open at birth. The degree of opening varied, but the observations were simply recorded as "all-or-none". The defect is probably caused by excessive bulging of the eye produced by certain changes in the skull.
2. Cranial blisters. These were slight swellings on the head which were usually accompanied by hemorrhages in the skin. The blisters are probably due to bulging of the brain through gaps in the roof of the cranium.

3. Vaulted cranium. This character is caused by the vaulting of the frontal bones resulting in a sharp bulge in the profile of the cranium.
4. Tail abnormalities. The main abnormalities recorded in this category were kinkiness and extremely shortened tails. A dose of 320r produced tails that were in many cases nothing more than stubs.
5. Digital abnormalities. Overgrowth and reduction in the number of digits were the most common type of abnormality observed. Reduction ranged from loss of a single digit either by actual loss or by fusion, to complete absence of differentiation resulting in a paddle-shaped appendage. In a few cases the entire limb was missing. Overgrowth, as used here, means the actual duplication of a digit or part of a digit and not merely the lengthening of a digit.

The experimental results are given in Table 4. In addition to the overall total, the data have been broken down into two sub-populations, one consisting of inbred progeny, the other of hybrid progeny. Emphasis was directed toward the detection of possible differences in response to <u>in utero</u> irradiation between inbred and hybrid progeny.

In Table 4, the column headed "Abnormal" includes the percentage of mice having at least one of the specified abnormalities. There is an obvious increase in the percentage

Table 4. Percentage of developmental abnormalities induced by irradiation at 10-1/2 days gestation

	Morphological characters observed at birth								
	80r			160r			320r		
	Inbred	Hybrid	Overall	Inbred	Hybrid	Overall	Inbred	Hybrid	Overall
Number of animals observed	72	76	148	22	48	70	17	33	50
Abnormal	7	3	5	64	29	40	100	100	100
Eyelids open	1	0	1	9	2	4	6	12	10
Cranial blister	0	1	1	14	2	6	100	85	90
Vaulted cranium	0	0	0	0	2	1	24	6	12
Abnormal tail	6	1	3	41	10	20	100	100	100
Overgrowth of feet									
Forefeet	0	0	0	5	0	1	0	0	0
Hind feet	0	0	0	23	10	14	12	15	14
Reduction of feet									
Forefeet	0	0	0	5	4	4	65	70	68
Hind feet	0	0	0	0	2	1	59	67	64

of abnormal mice in going from 80r to 160r and again from 160r to 320r, a dose of 320r causing all progeny to be abnormal to some degree. It is not included in the table, but it was noted that whereas none of the mice treated with 80r had more than one abnormality, and only about 15 per cent treated with 160r had more than one abnormality, all of the progeny exposed to 320r had two or more abnormalities.

Digital abnormalities were restricted to 160 and 320r. The overall incidence of overgrowth remained approximately the same with both doses (16 per cent and 14 per cent respectively). However, a distinct difference in response between the forefeet and hind feet was shown. Only one case (with 160r) of overgrowth was found in the forefeet, while all remaining cases of overgrowth were observed in the hind feet.

The incidence of reduction is slight after a dose of 160r (6 per cent), but increases to 66 per cent after 320r. The difference in response between fore and hind feet is not significant.

A comparison of the response of the different genotypes to the induction of abnormalities is most revealing with the dose of 160r, since with 320r all progeny were affected, and with 80r, the percentage of abnormal animals was too small with the actual number of animals observed to detect a difference. After a dose of 160r, however, a distinct difference in the response of inbred and hybrid progeny becomes evident.

Whereas 64 per cent of the inbred progeny show some developmental abnormality, only 29 per cent of the hybrid progeny do. This distribution of abnormalities is significantly different from that expected if both populations had similar responses to the treatment ($\chi^2 = 6.10$; $.01 < P < .05$).

Effects of In Utero Irradiation upon Prenatal Viability

Proportion of females giving birth to litters at the expected end of gestation

In this study the pregnancy of females that were to be irradiated at 6-1/2 and 10-1/2 days was timed solely from the appearance of a vaginal plug. Since this plug is not an absolute proof of a successful pregnancy, failure of a female to give birth to a litter when irradiated at either of these stages could therefore have been due to either:

1. Failure of conception to have taken place.
2. Prenatal loss of the entire litter.

In the case of females due to be irradiated at 14-1/2 and 17-1/2 days an external diagnosis of pregnancy was also made. If a female was adjudged not to be pregnant upon external observation, she was not irradiated. None of the females determined not to be pregnant by this method gave birth to a litter. Five females believed to be pregnant at 14-1/2 days did not yield any litters. These cases will be discussed later.

The data are summarized in Table 5.

Table 5. Number of females delivering young at term

Dose	Number of females irradiated	Number bearing litters	Per cent
	Irradiation at 6-1/2 days		
20r	18	12	67
80r	23	14	61
160r	20	14	70
320r	9	0	0
	Irradiation at 10-1/2 days		
20r	19	14	74
80r	26	18	69
160r	13	10	77
320r	40	13	33

The most significant things to be observed in this table are the results of irradiation with 320r. None of the females given 320r at 6-1/2 days gave birth to a litter indicating the following possibilities:

1. None of the 9 females were actually pregnant at the time of irradiation.
2. 320r caused a 100 per cent prenatal loss of progeny.
3. Some combination of 1. and 2.

Females exposed to 20, 80 or 160r at 6-1/2 or 10-1/2 days produced litters approximately 70 per cent of the time, indicating that some 30 per cent of the females in which a plug

was observed were not actually pregnant. Females irradiated with 320r at 10-1/2 days yielded litters in only 33 per cent of the cases, an amount differing significantly from the overall 70 per cent in the other treatments. It appears that this treatment also may cause 100 per cent prenatal loss of some litters. The possibility that some of these losses may have been due to loss after parturition is discussed in the following section.

A comparison of the two general hereditary types, inbreds and hybrids, used in this experiment reveals that there is not a differential response to *in utero* irradiation as measured by prenatal loss of entire litters. Of the 40 females given 320r at 10-1/2 days, 4 of 18 inbred females (22 per cent) and 9 of 22 hybrid females (41 per cent) had litters. The difference of 19 per cent is not significant, however ($\chi^2 = 0.83$; $.50 < P < .25$).

Mean size of litters brought to term

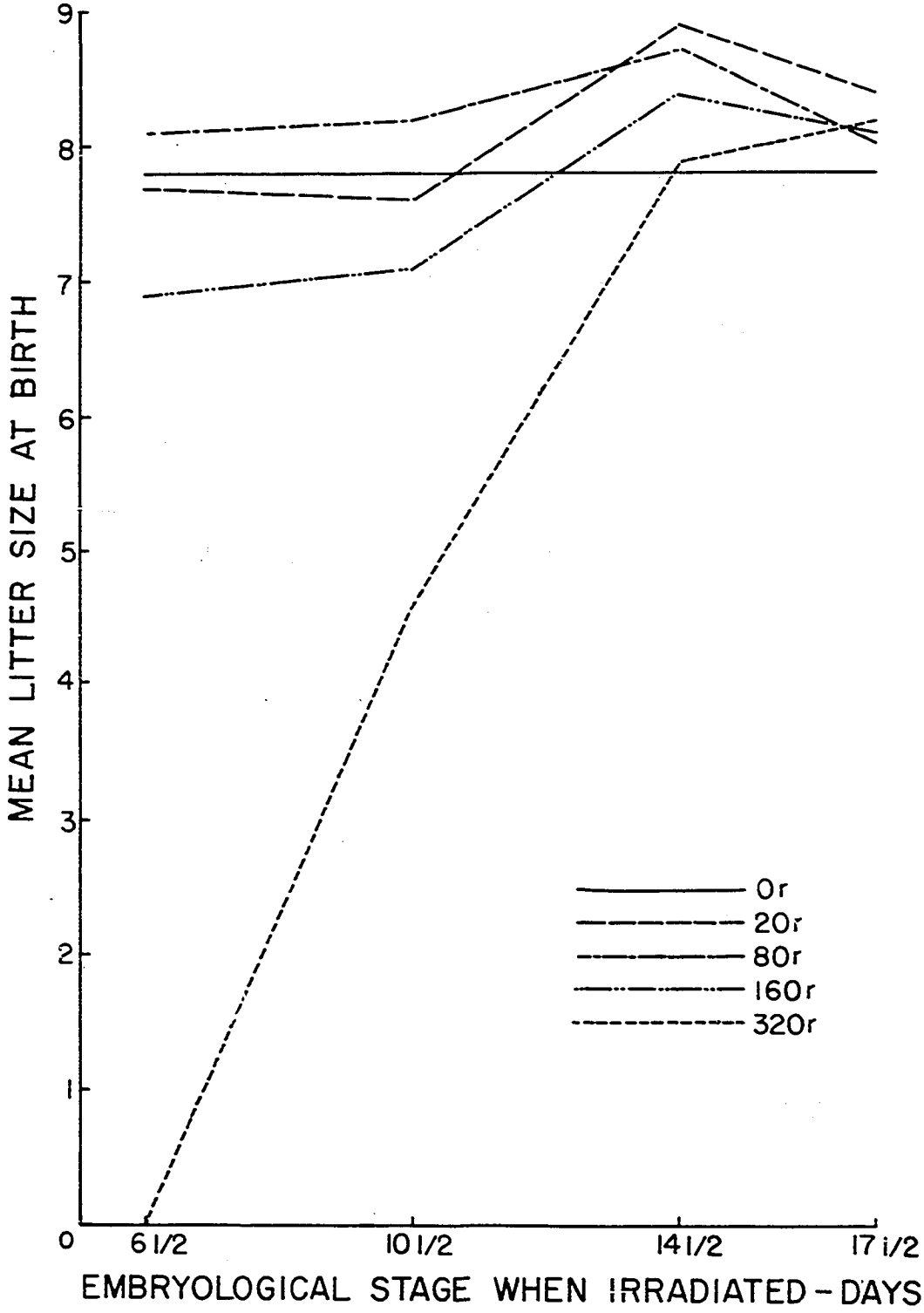
Prenatal viability may also be measured by the number of progeny surviving to term. The mean litter size for each treatment is shown in Table 6 and Figure 2. Due to the small number of litters in each treatment the standard errors of the means tend to be high so that small differences between means are regarded as being insignificant. The only treatment that differs significantly from the controls is 320r at 10-1/2 days gestation. This treatment caused the average litter size to

Table 6. Means and standard errors of litter sizes at birth after *in utero* radiation

Dose	Embryological age	Number litters	Number young	Mean, std. error
0r		13	101	7.8 ± .6
20r	6-1/2 days	12	92	7.7 ± .4
	10-1/2 days	14	106	7.6 ± .7
	14-1/2 days	13	116	8.9 ± .7
	17-1/2 days	16	134	8.4 ± .5
80r	6-1/2 days	14	114	8.1 ± .3
	10-1/2 days	18	148	8.2 ± .5
	14-1/2 days	14	122	8.7 ± .5
	17-1/2 days	11	88	8.0 ± .7
160r	6-1/2 days	14	97	6.9 ± .6
	10-1/2 days	10	71	7.1 ± 1.0
	14-1/2 days	20	167	8.4 ± .5
	17-1/2 days	14	113	8.1 ± .6
320r	10-1/2 days	13	60	4.6 ± .5
	14-1/2 days	9	71	7.9 ± .5
	17-1/2 days	24	197	8.2 ± .4

be reduced to 4.6 compared to 7.8 for the controls, a loss of 41 per cent. It has already been mentioned that all of the progeny born after treatment with 320r at 10-1/2 days were grossly abnormal morphologically, and, in addition, were all dead at the time of recording of the birth of the litter. Some of the progeny had also been partly eaten by the dam. It is possible that the decreased litter size with this treatment was not due to prenatal death and subsequent resorption, but

Figure 2. Mean size of litters brought to term by irradiated females.


MEAN LITTER SIZE AT BIRTH
9
8
7
6
5
4
3
2
1
0
6 1/2
10 1/2
14 1/2
17 1/2
EMBRYOLOGICAL STAGE WHEN IRRADIATED - DAYS
0r
20r
80r
160r
320r

may have been due to the eating of abnormal young by the dam. An additional number of animals were given 320r at 10-1/2 days in order to examine this possibility. Pregnant females were killed at 17-1/2 days gestation, and the contents of the uteri examined. It was found that of 68 embryos from 9 litters all but two of them were already dead at the time of observation. Of the dead embryos, half of them were dead quite soon after treatment as shown by the almost complete resorption and the lack of distinguishable features of the embryos. In one of the litters all of the embryos were almost completely resorbed indicating that this treatment may cause a 100 per cent prenatal loss of progeny.

There is one treatment, 160r at 6-1/2 days which, although it is not significantly lower than controls, should be considered further. Irradiation during preimplantation stages has been reported to decrease litter size. It is likely that irradiation at this early post-implantation stage may also cause a reduction in litter size due to loss of individuals within litters. A dose of 320r at 6-1/2 days was seen to have evidently caused a complete termination of pregnancies.

Neonatal mortality

The percentage of progeny born dead in the overall mouse population following irradiation in utero reaches a maximum of 100 per cent on day 10-1/2 after a dose of 320r (Table 7 and

Figure 3). The embryological stage of 10-1/2 days appears to be most sensitive to neonatal mortality. The LD_{50} at birth is somewhere between 80 and 160r, the former causing only 5 per cent stillborns, the latter 76 per cent. The only other treatment that appreciably increases neonatal mortality is 320r at 14-1/2 days, when 23 per cent of the progeny are born dead. Doses of 20r or 80r appear to be ineffective in increasing neonatal mortality when given at any embryological age.

A differential response of the inbred and hybrid progeny is evident following a dose of 160r at 10-1/2 days (Table 7, Figure 4). Mortality was 100 per cent in the inbreds but only 64 per cent in the hybrids. ($\chi^2 = 8.83$; $.01 < P < .001$). The differences between inbreds and hybrids in all other treatments are not significant. However, after 320r at 14-1/2 days, which did cause an increase in mortality, the data support the contention that the inbreds were more sensitive than the hybrids.

Effects of *In Utero* Irradiation and Irradiation at Term upon Growth

In the results that follow the two sexes are treated separately in accordance with the generally observed fact that male mice grow more rapidly than females. In interpreting the data it was also necessary to take into account the fact that growth in the mouse is known to be affected by

Table 7. Percentage incidence of stillborn births

Embryological age	Dose	Inbreds Number observed	Inbreds Per cent	Hybrids Number observed	Hybrids Per cent	Overall Number observed	Overall Per cent
	0r	23	4	78	1	101	2
6-1/2 days	20r	33	15	59	2	92	7
	80r	31	10	83	4	114	5
	160r	32	6	65	12	97	10
10-1/2 days	20r	39	13	67	3	106	7
	80r	72	6	76	4	148	5
	160r	23	100	48	64	71	76
	320r	18	100	42	100	60	100
14-1/2 days	20r	38	3	78	8	116	6
	80r	53	2	69	3	122	2
	160r	83	1	84	4	167	2
	320r	20	30	51	20	71	23
17-1/2 days	20r	61	5	73	0	134	2
	80r	24	4	70	0	94	1
	160r	39	18	74	5	113	10
	320r	89	15	108	3	197	8

Figure 3. Percentage incidence of stillborn births in the overall mouse population following *in utero* irradiation.

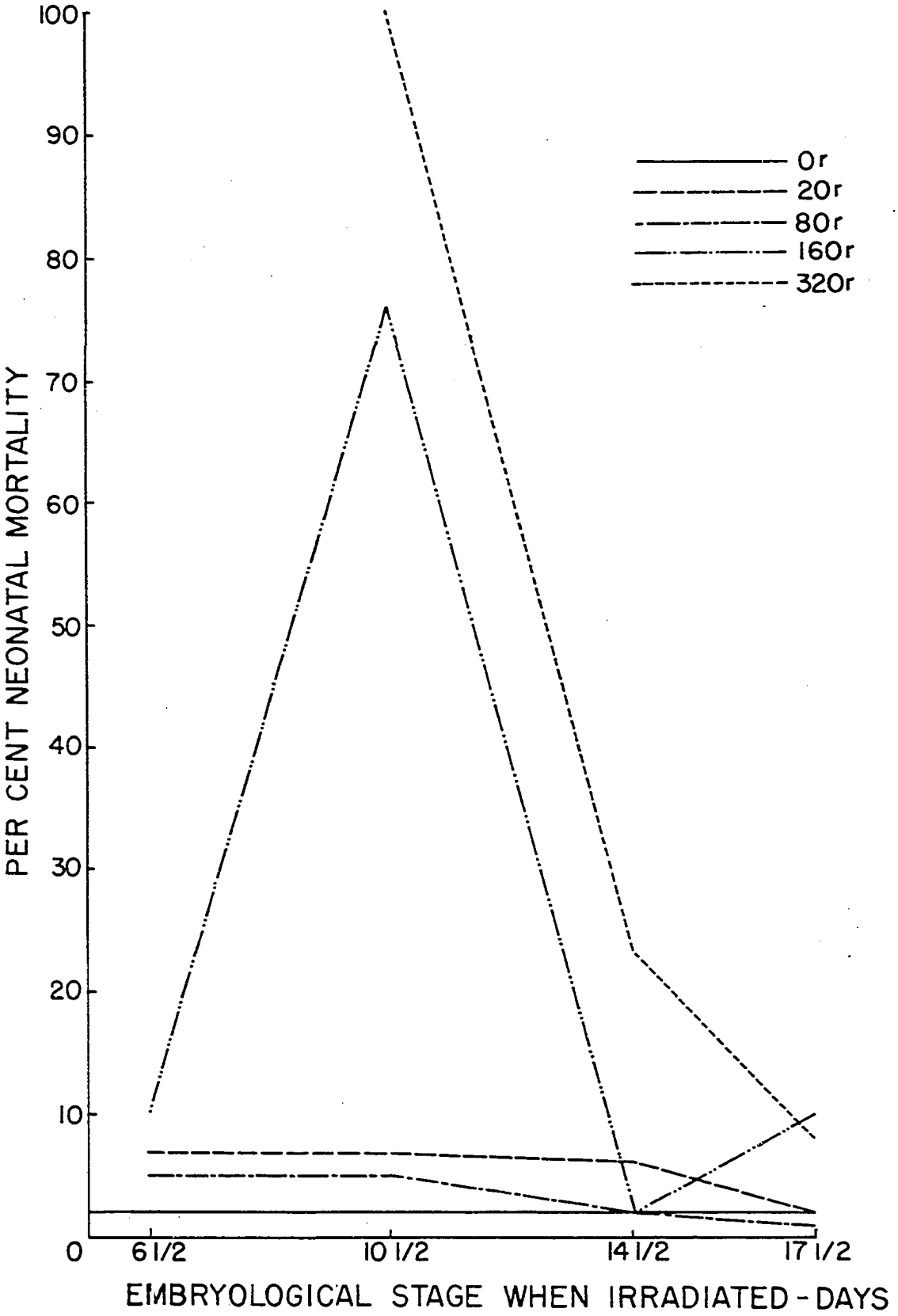
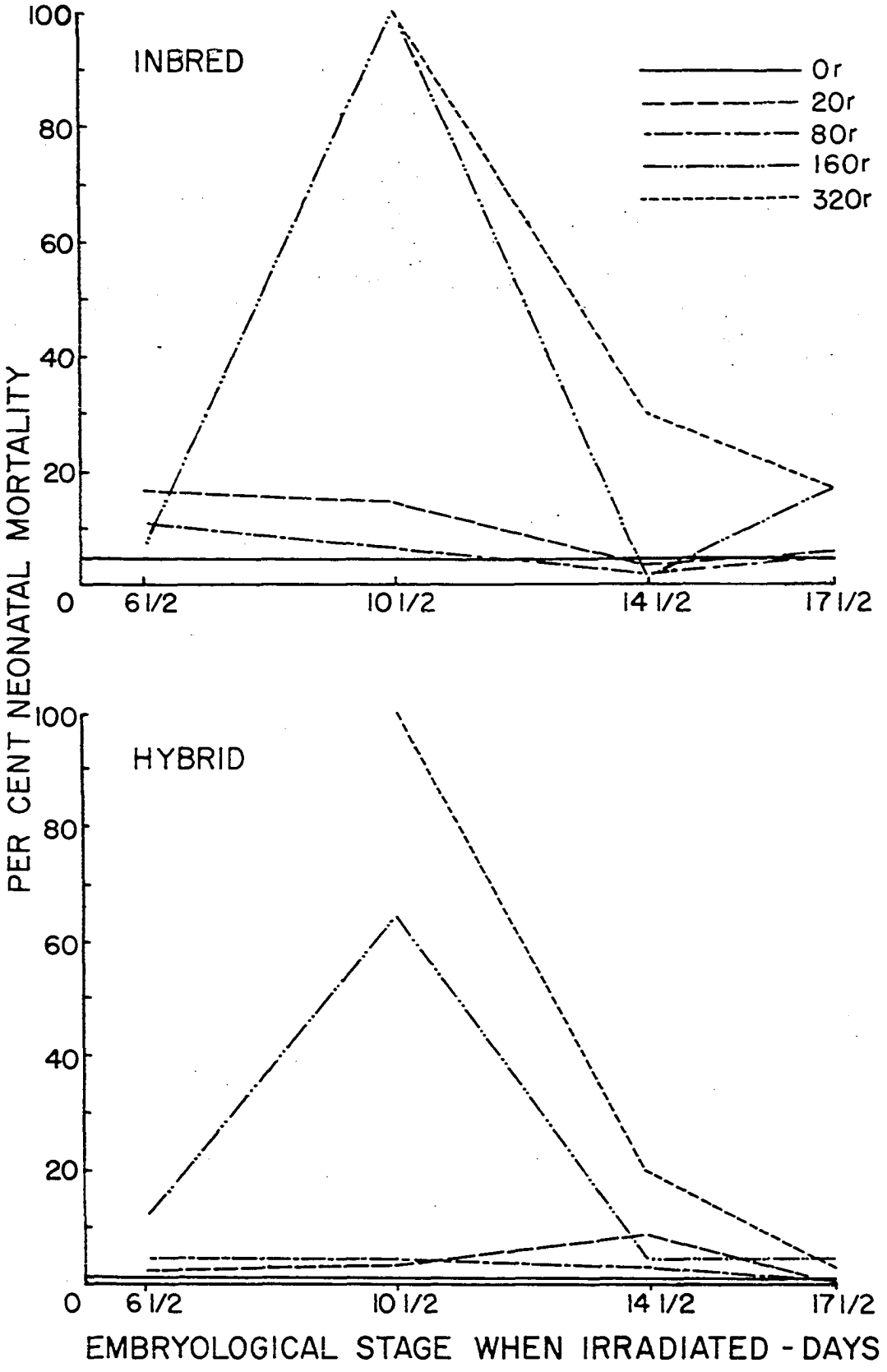

100
90
80
70
60
50
40
30
20
10
0
PER CENT NEONATAL MORTALITY
0r
20r
80r
160r
320r
6 1/2
10 1/2
14 1/2
17 1/2
EMBRYOLOGICAL STAGE WHEN IRRADIATED - DAYS

Figure 4. Percentage incidence of stillborn births among inbred and hybrid progeny.


INBRED
HYBRID
0r
20r
80r
160r
320r
100
80
60
40
20
0
6 1/2
10 1/2
14 1/2
17 1/2
PER CENT NEONATAL MORTALITY
EMBRYOLOGICAL STAGE WHEN IRRADIATED - DAYS

litter size, individuals from smaller litters in general tending to grow faster than individuals from larger litters. The body weight data in this study bore this out, and all weights from birth to 75 days were adjusted for litter size at birth. The regressions of body weights on litter size at birth were calculated for each treatment. Although there was some heterogeneity between the regression coefficients of all the treatments, it was felt that the mean regression coefficient represented the best correction to be used in adjusting the body weights. A litter size of 9, which represents the mean over all treatments, was used as the base point in adjusting for litter size, individuals from litters larger than 9 having a factor added. The actual amounts of the corrections used are included in the Appendix.

Concerning the genotypes of the mice used in this study, the growth results have been examined several ways. By pooling mice of different genotypes into one population, a single "overall" population has been obtained and the data interpreted as to the effects on this overall population. Additional analyses have been made by dividing this overall population into 2 sub-populations, one consisting of inbred progeny and one of hybrid progeny. The influence of different genotypes among the inbreds and hybrids are considered in the section on components of variation.

In addition to genotype the 2 main factors affecting

growth in this experiment were the level of irradiation and the embryological age of mice at the time of irradiation. It has been necessary therefore to examine the data by considering both of these factors. The results are most clearly interpreted by examining each of these factors separately. Within each sex the results are discussed first by a consideration of the overall effects of each level of irradiation over all embryological ages, and then by a consideration of the effects observed with different levels of irradiation within each embryological age separately.

The mean body weights and standard errors of the means have been calculated within each dose-embryological age treatment combination separately. Although the frequencies within each of the cells are random variables, they have been treated as if they were actually fixed.

Radiation response of males

Influence of embryological age at irradiation upon growth.

Birth weights Inspection of the body weight means and standard errors of the means in Table 8 reveals that at doses of 80r or greater differences in response of the embryological ages become evident. Within these doses, 10-1/2 days appears to be the most sensitive, birth weights ranging from 89 per cent of control weights after 80r to only 44 per cent of control weights after a dose of 320r. A dose of 80r given at

any age other than 10-1/2 days is apparently ineffective in changing birth weights since the differences are well within the limits of sampling error. However, after doses of 160r or 320r effects are also found after irradiation at 14-1/2 days. The decrease is considerably less than with a similar dose at 10-1/2 days. A dose of 320r at 14-1/2 days has an effect similar to a dose of 160r at 10-1/2 days.

12 Day weights The high sensitivity of the 10-1/2 day embryo is further shown by the 100 per cent mortality shortly after birth in animals that had received 160r or 320r. At 12 days a definite weight decrease is evident only in those progeny that had been given 320r. Within this dose both 17-1/2 day and newborn progeny are less than controls (82 and 84 per cent respectively), and 14-1/2 day progeny are also probably less, although the difference is not statistically significant as there is a large amount of variation due to the small number of observations, most of the progeny in this group having died shortly after birth. Treatment with 160r at 6-1/2 days seemed to have resulted in a definite weight increase over controls at 12 days.

26 Day weights At 26 days of age significant differences in body weights can be observed after doses of 160r or 320r. Within both of these doses 14-1/2 days is most affected. With 320r progeny from all embryological ages weigh less than controls, but with 160r, 17-1/2 day and

Table 8. Males - body weight means, adjusted for litter size at birth

Dose	Embryological age	Age post-parturition		
		Birth	12 Days	26 Days
0r		1.40 ± .02	5.0 ± .1	8.9 ± .4
0r*		1.35 ± .02	5.3 ± .1	9.5 ± .3
20r	6-1/2 days	1.36 ± .02	5.3 ± .2	9.7 ± .4
	10-1/2 days	1.39 ± .02	5.5 ± .1	10.8 ± .3
	14-1/2 days	1.37 ± .01	4.9 ± .1	9.3 ± .3
	17-1/2 days	1.39 ± .01	5.1 ± .1	9.2 ± .3
	Newborn	1.37 ± .01	4.7 ± .2	9.1 ± .4
80r	6-1/2 days	1.36 ± .02	5.5 ± .2	10.2 ± .4
	10-1/2 days	1.25 ± .01	5.1 ± .1	9.1 ± .3
	14-1/2 days	1.31 ± .02	4.9 ± .2	8.9 ± .3
	17-1/2 days	1.35 ± .02	5.0 ± .2	9.2 ± .6
	Newborn	1.36 ± .01	5.3 ± .1	9.3 ± .3
160r	6-1/2 days	1.35 ± .02	6.1 ± .2	11.3 ± .4
	10-1/2 days	0.97 ± .02	---	---
	14-1/2 days	1.25 ± .02	4.9 ± .1	7.3 ± .3
	17-1/2 days	1.33 ± .02	4.7 ± .1	8.4 ± .4
	Newborn	1.35 ± .01	4.7 ± .2	8.3 ± .4
320r	10-1/2 days	0.61 ± .02	---	---
	14-1/2 days	0.98 ± .02	4.1 ± .5	5.8 ± .6
	17-1/2 days	1.32 ± .02	4.1 ± .1	6.6 ± .4
	Newborn	1.37 ± .01	4.2 ± .2	6.6 ± .3
		40 Days	60 Days	75 Days
0r		17.7 ± .4	23.1 ± .3	24.7 ± .3
0r*		17.9 ± .4	23.7 ± .3	25.4 ± .3
20r	6-1/2 days	19.1 ± .4	23.8 ± .4	25.9 ± .4
	10-1/2 days	20.1 ± .3	24.6 ± .3	25.9 ± .2
	14-1/2 days	18.7 ± .3	23.4 ± .3	24.7 ± .3
	17-1/2 days	18.0 ± .6	23.7 ± .4	25.3 ± .4
	Newborn	17.9 ± .6	22.6 ± .4	24.1 ± .4

*Represents the group of controls used in that part of the experiment dealing with irradiation of newborn animals.

Table 8. (Continued)

Dose	Embryological age	Age post-parturition		
		40 Days	60 Days	75 Days
80r	6 days	19.2 ± .5	24.1 ± .3	25.9 ± .3
	10 days	18.1 ± .3	21.7 ± .3	23.0 ± .3
	14 days	17.4 ± .7	22.2 ± .6	23.6 ± .6
	17 days	17.5 ± .6	23.2 ± .4	24.6 ± .4
	Newborn	17.8 ± .4	22.1 ± .3	23.6 ± .3
160r	6 days	20.1 ± .5	24.6 ± .4	26.7 ± .4
	10 days	---	---	---
	14 days	14.5 ± .4	19.7 ± .4	21.3 ± .3
	17 days	16.0 ± .6	21.0 ± .3	22.1 ± .3
	Newborn	15.3 ± .7	20.1 ± .8	21.7 ± .6
320r	10 days	---	---	---
	14 days	7.6 ±1.0	8.8 ± .1	9.1 ± .2
	17 days	12.2 ± .7	17.1 ± .6	18.6 ± .5
	Newborn	12.4 ± .5	16.8 ± .5	18.8 ± .5

newborn progeny are not significantly different from controls. The advantage of the progeny from 160r at 6-1/2 days over controls is continued at 26 days. In addition, progeny from 20r at 10-1/2 days are heavier than controls.

40 Day weights The same general response is evident at 40 days as existed at 26 days. Newborn progeny irradiated with 160r now weigh less than controls, and progeny from 160r at 17-1/2 days are not quite significantly lower than controls. Progeny from 320r at 14-1/2 days are relatively even lighter than controls than at 26 days, weighing only 43 per cent of

controls compared to 65 per cent at 26 days.

60 Day weights Differences in sensitivity between embryological ages become apparent after a dose as low as 80r at the 60 day observation. At that time progeny from 80r at 10-1/2 days weigh slightly less than controls. A dose of 80r at other ages still apparently has no effect on body weight. Newborn progeny given 80r, however, now weigh less than their own simultaneous controls. Progeny irradiated at 17-1/2 days with 160r weigh less than controls for the first time.

75 Day weights The relative sensitivities of the embryological ages within each dose remain approximately the same as at 60 days. It is evident that the treatments producing significantly lower body weights than controls have not recovered much of the weight loss by 75 days as the following table, which includes those treatments that caused a significant decrease in body weight, shows.

Table 9. Males - ratio of the treated body weight means to control body weight means

		Treated/Control					
Dose	Embryological age	Age in days Birth	12	26	40	60	75
80r	10-1/2 days	.89	1.02	1.02	1.02	.94	.93
160r	Newborn	.96	.89	.87	.85	.85	.85
160r	17-1/2 days	.95	.94	.94	.90	.91	.89
160r	14-1/2 days	.89	.96	.82	.82	.85	.86
320r	17-1/2 days	.94	.82	.74	.69	.74	.75
320r	Newborn	.98	.79	.69	.69	.71	.74
320r	14-1/2 days	.70	.80	.65	.43	.38	.37

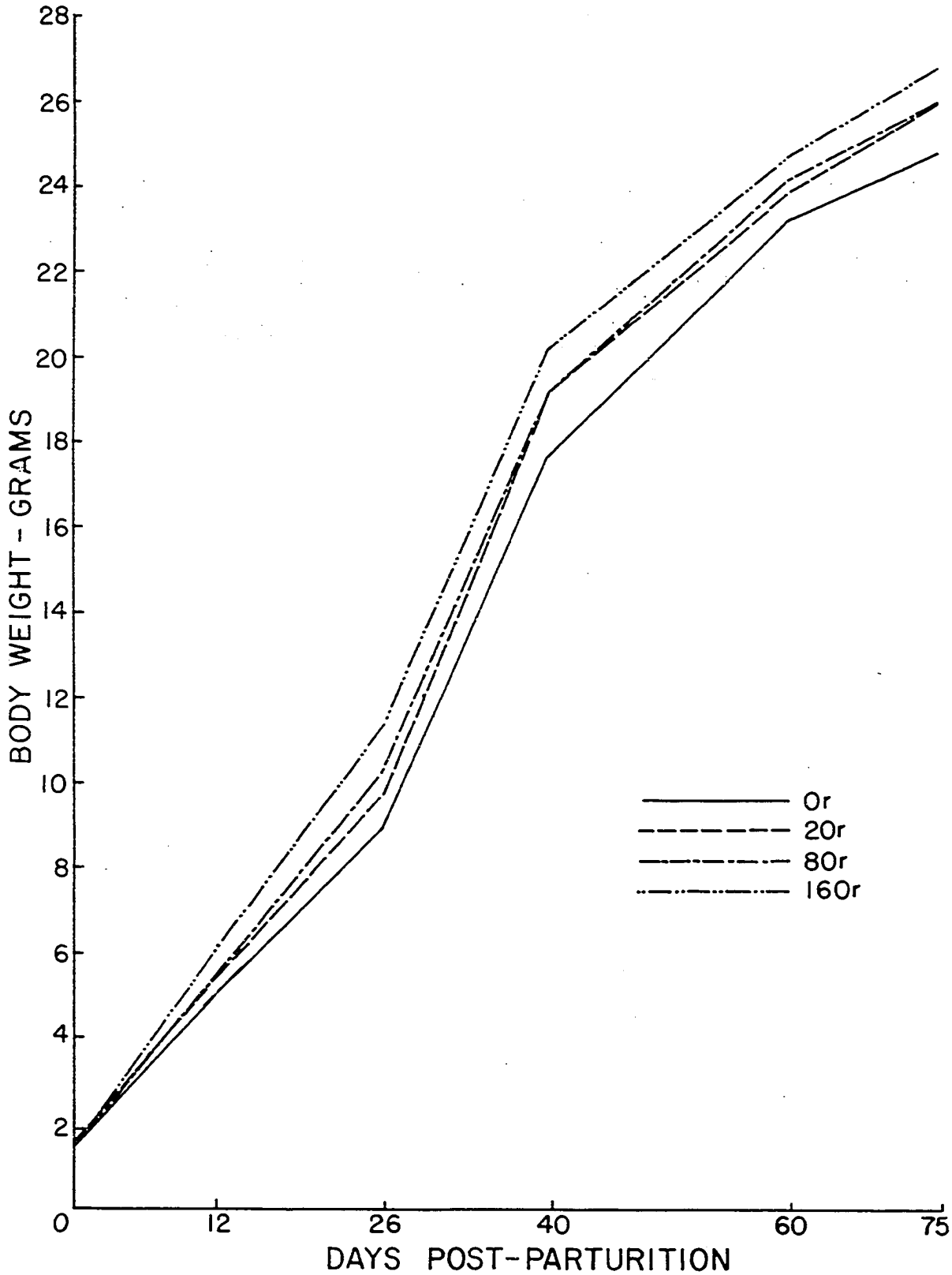
In general the minimum ratio in each treatment occurs around the 40 day observation. It would appear from the trends of these ratios that the lower weight gain will not be made up.

Influence of level of irradiation within each embryological age

Irradiation at 6-1/2 days (Figure 5) Irradiation at 6-1/2 days with doses up to 160r has no deleterious effect on postnatal growth. Embryos that had been irradiated with 160r even showed an accelerated growth rate compared to controls. A significant difference in body weights was observed by the 12th day, and the difference continued throughout the remainder of the period of observations. The relative difference reached a maximum at 26 days (27 per cent) and appeared to be leveling off at about 8 per cent at 75 days.

Irradiation at 10-1/2 days (Figure 6) Doses of 80r and above have noticeable effects on growth when given at 10-1/2 days, resulting in lowered body weights at birth. After a dose of 320r birth weights were less than half of those of controls. All of the progeny that had received 160r or 320r on 10-1/2 days did not survive beyond a few days after birth. Progeny that had received 20r weighed more than controls from the 12th day on. Although animals that had been given 80r weighed less at birth than controls, the survivors could not be distinguished from controls until 60

Figure 5. Irradiation at 6-1/2 days. Males - body weight means, adjusted for litter size at birth.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12
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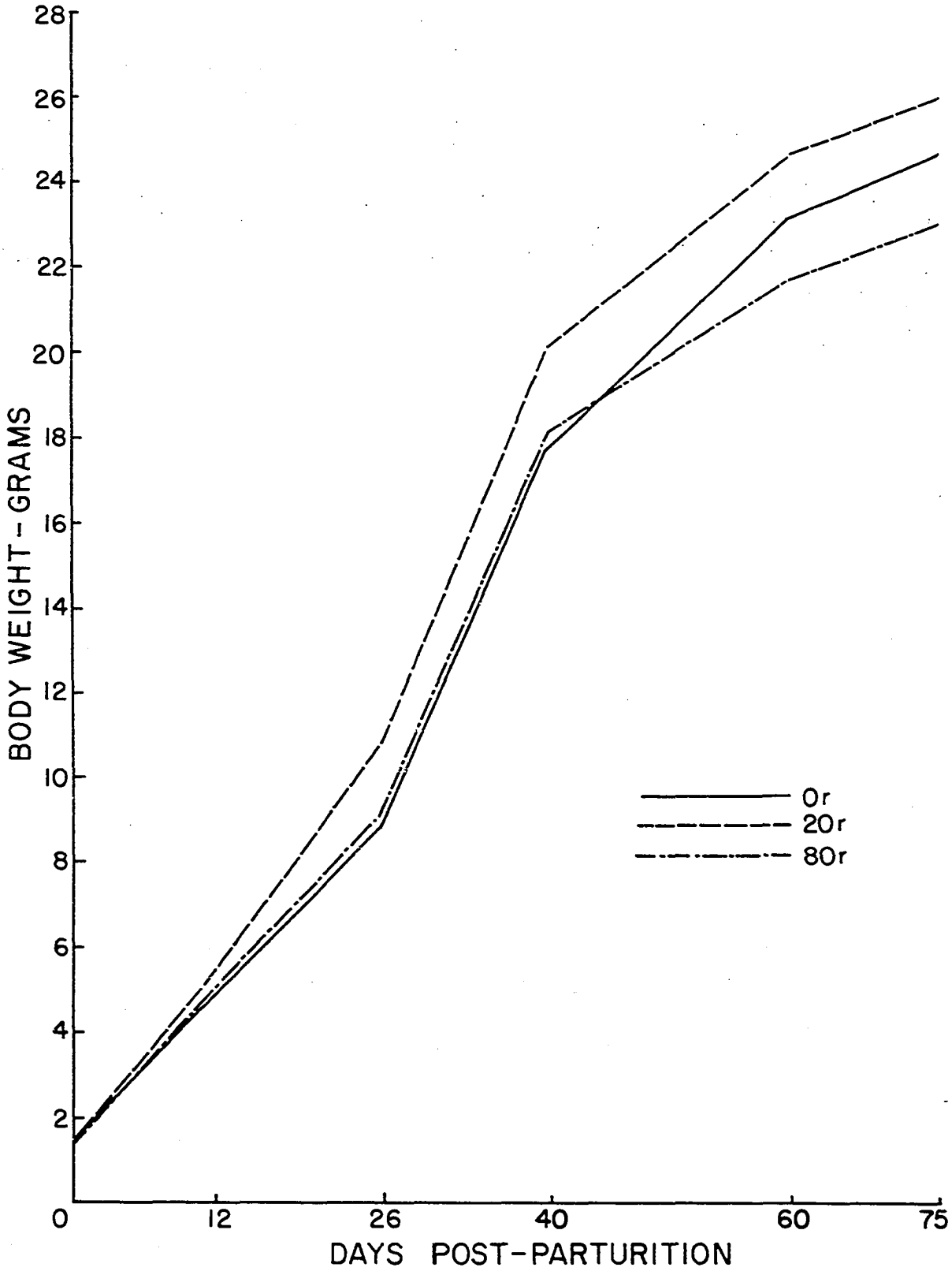
BODY WEIGHT - GRAMS
12
26
40
60
75
DAYS POST-PARTURITION
0r
20r
80r
160r

Figure 6. Irradiation at 10-1/2 days. Males - body weight means, adjusted for litter size at birth.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12
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40
60
75
BODY WEIGHT - GRAMS
DAYS POST-PARTURITION
0r
20r
80r

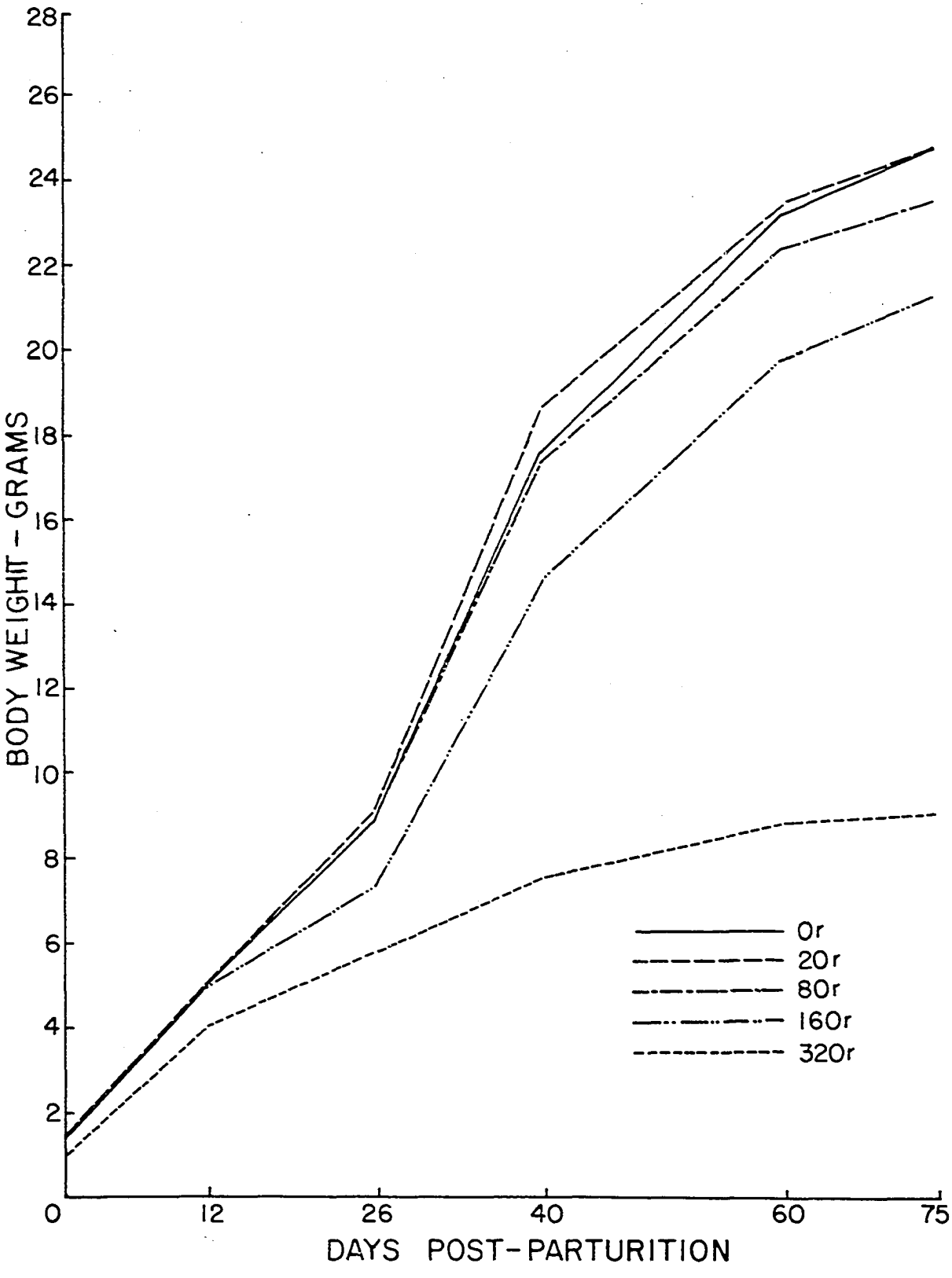
days after birth. At 75 days animals in this treated group were 7 per cent lower than controls.

Irradiation at 14-1/2 days (Figure 7) Differences in birth weights are apparent after irradiation with 160r or 320r at 14-1/2 days. However, by the 12 day observation all irradiated groups were indistinguishable from controls. There were only a few surviving progeny in the 320r group. By 26 days of age animals that had received 160r and 320r were less than controls. This pattern holds throughout the rest of the period of observation, 160r progeny weighing 89 per cent and 320r progeny only 37 per cent of controls at 75 days.

Irradiation at 17-1/2 days (Figure 8) Following irradiation at 17-1/2 days there is no significant effect on birth weights. By 12 days 320r progeny were lower than controls and remained lower through 75 days of age, reaching a minimum at 40 days when they were only 68 per cent of control weights, and recovering to only 75 per cent by 75 days. A difference between 160r animals and controls is detectable at 60 days and continues to 75 days. Doses of 20r or 80r apparently did not produce significant weight changes at any period.

Irradiation at birth (Figure 9) The birth weights of all "irradiated" groups actually represent controls since all progeny were weighed before irradiation. Response of animals

Figure 7. Irradiation at 14-1/2 days. Males - body weight means, adjusted for litter size at birth.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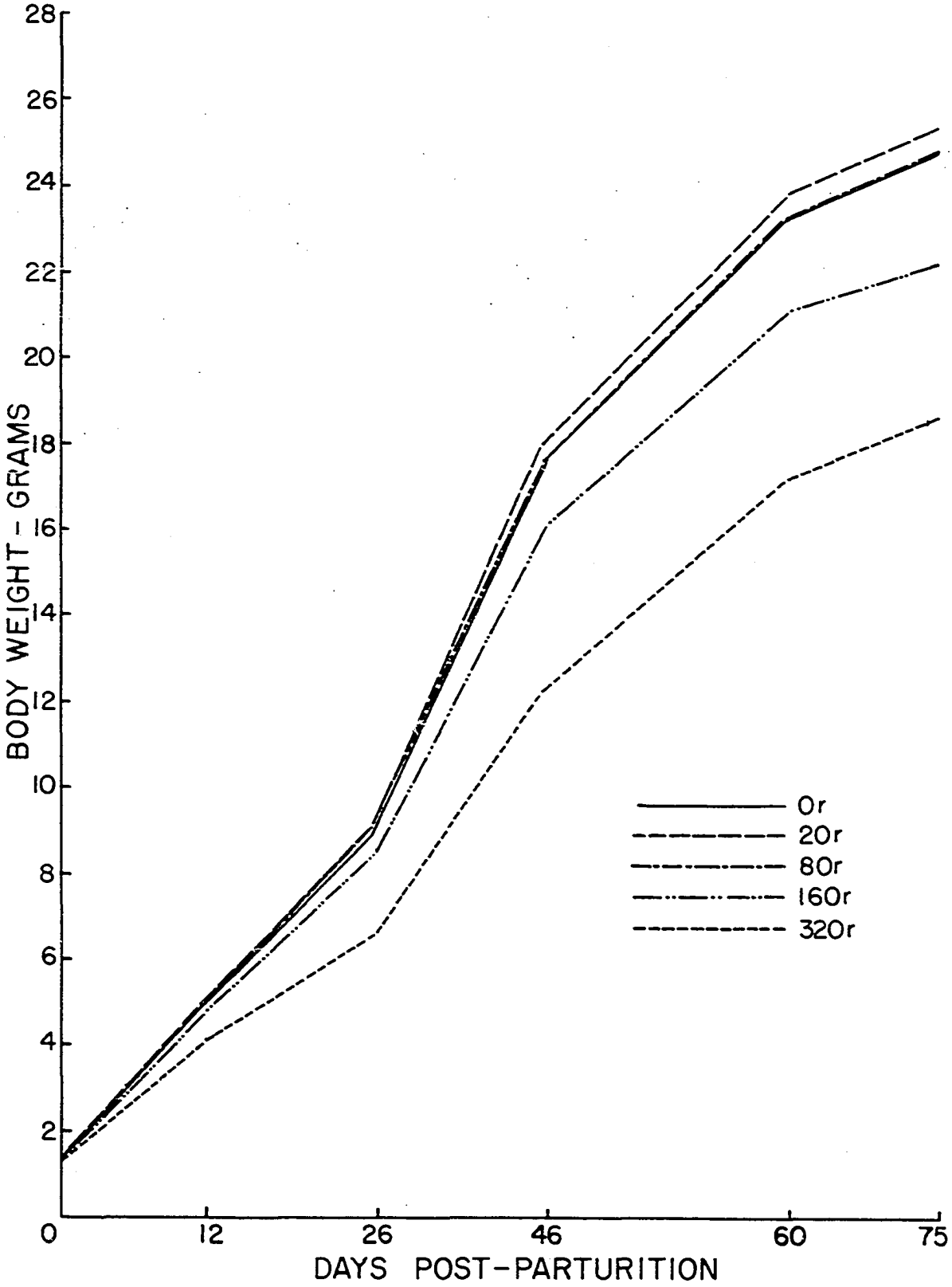
60
75
BODY WEIGHT - GRAMS
DAYS POST-PARTURITION
0r
20r
80r
160r
320r

Figure 8. Irradiation at 17-1/2 days. Males - body weight means, adjusted for litter size at birth.


BODY WEIGHT - GRAMS
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0
12
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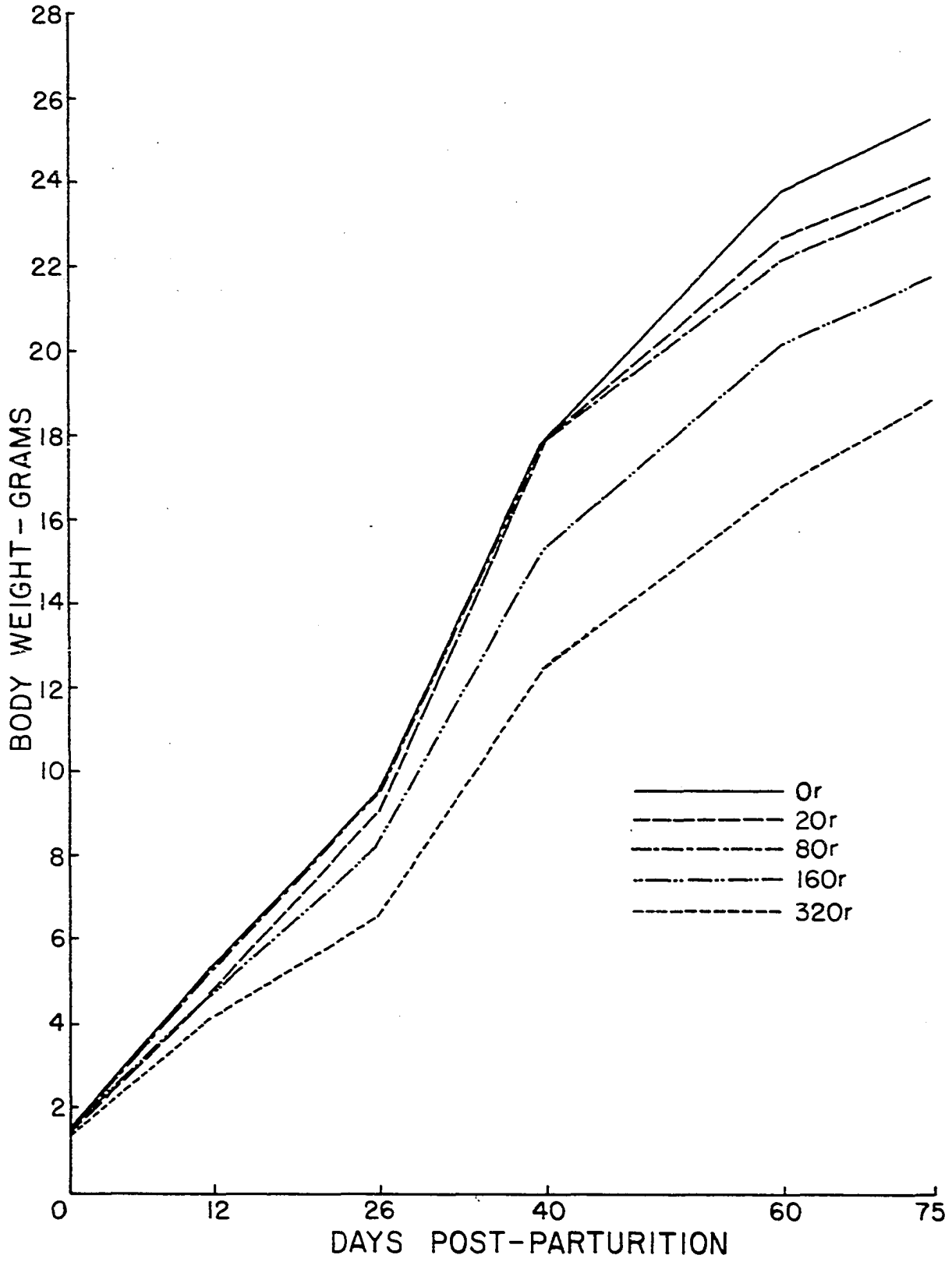
46
60
75
DAYS POST-PARTURITION
0r
20r
80r
160r
320r

Figure 9. Irradiation of newborn mice. Males - body weight means, adjusted for litter size at birth.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75
BODY WEIGHT - GRAMS
DAYS POST-PARTURITION
0r
20r
80r
160r
320r

irradiated at birth follows closely the weight response following irradiation at 17-1/2 days. Animals given 160r at birth are significantly lower than controls by 26 days. Further evidence that animals irradiated at birth are slightly more sensitive to growth changes than animals irradiated at 17-1/2 days is shown by the fact that after a dose of 80r to newborn progeny, a significant weight change was observed by 60 days of age and was continued through 75 days.

Radiation response of females

The mean body weights of the females are shown in Table 10. Examination of these results shows that the general response pattern of the females is similar to that of the males. For the birth weights doses of 80r or greater may produce a noticeable change. An embryological age of 10-1/2 days is the most sensitive to weight change. At 75 days of age the same treatments that produced significantly lower body weights in the males have produced significantly lower body weights in the females. These treatments were 80r at 10-1/2 days, 160r at 14-1/2 days and 17-1/2 days, 160r at term, 320r at 14-1/2 and 17-1/2 days and 320r at term. The relative differences of these changes expressed as the ratio of treated to control weights are shown in Table 11.

As in the males, the minimum of these ratios, particularly in those treatments affecting growth most severely, occurs around the 40 day observation. The actual sizes of

Table 10. Females - body weight means, adjusted for litter size at birth

Dose	Embryological age	Age post-parturition		
		Birth	12 Days	26 Days
Or		1.32 ± .01	4.9 ± .1	8.0 ± .3
Or*		1.30 ± .02	5.3 ± .1	9.5 ± .4
20r	6-1/2 days	1.28 ± .03	5.4 ± .2	10.0 ± .4
	10-1/2 days	1.33 ± .02	5.5 ± .1	10.1 ± .3
	14-1/2 days	1.32 ± .01	4.8 ± .1	8.7 ± .2
	17-1/2 days	1.36 ± .01	5.2 ± .1	9.7 ± .3
	Newborn	1.32 ± .01	4.8 ± .2	8.5 ± .3
80r	6-1/2 days	1.35 ± .01	5.1 ± .1	8.9 ± .3
	10-1/2 days	1.21 ± .01	5.3 ± .2	9.0 ± .3
	14-1/2 days	1.31 ± .02	4.9 ± .1	9.2 ± .2
	17-1/2 days	1.31 ± .02	5.2 ± .2	9.2 ± .4
	Newborn	1.33 ± .01	4.9 ± .1	9.1 ± .3
160r	6-1/2 days	1.23 ± .02	5.8 ± .2	10.8 ± .4
	10-1/2 days	0.97 ± .02	---	---
	14-1/2 days	1.15 ± .01	4.4 ± .1	6.3 ± .2
	17-1/2 days	1.30 ± .02	4.8 ± .1	8.4 ± .4
	Newborn	1.34 ± .01	4.8 ± .2	8.2 ± .4
320r	10-1/2 days	0.57 ± .02	---	---
	14-1/2 days	0.96 ± .01	5.5 ± .2	6.8 ± .1
	17-1/2 days	1.25 ± .01	3.9 ± .1	6.4 ± .3
	Newborn	1.32 ± .01	4.3 ± .1	6.5 ± .2
		40 Days	60 Days	75 Days
Or		15.9 ± .4	19.9 ± .3	21.1 ± .3
Or*		16.6 ± .4	19.9 ± .3	20.8 ± .4
20r	6-1/2 days	16.9 ± .6	20.7 ± .3	21.9 ± .3
	10-1/2 days	17.9 ± .3	20.9 ± .4	22.0 ± .3
	14-1/2 days	15.8 ± .2	19.0 ± .2	20.6 ± .2
	17-1/2 days	17.1 ± .3	20.2 ± .2	21.8 ± .2
	Newborn	15.9 ± .3	18.9 ± .3	19.6 ± .4

*Represents the group of controls used in that part of the experiment dealing with irradiation of newborn animals.

Table 10. (Continued)

Dose	Embryological age	Age post-parturition		
		40 Days	60 Days	75 Days
80r	6 days	16.4 ± .3	20.0 ± .2	21.5 ± .2
	10 days	15.8 ± .3	18.4 ± .4	19.6 ± .4
	14 days	16.5 ± .3	19.6 ± .3	20.5 ± .3
	17 days	16.4 ± .4	20.0 ± .3	21.0 ± .3
	Newborn	15.7 ± .3	18.4 ± .3	19.6 ± .3
160r	6 days	17.9 ± .5	21.2 ± .5	21.6 ± .4
	10 days	---	---	---
	14 days	12.0 ± .3	15.5 ± .3	16.6 ± .3
	17 days	14.4 ± .4	17.8 ± .3	18.8 ± .3
	Newborn	14.5 ± .4	16.8 ± .3	17.8 ± .3
320r	10 days	---	---	---
	14 days	8.9 ± .9	10.5 ± .6	12.0 ± .0
	17 days	10.5 ± .5	14.4 ± .5	15.7 ± .5
	Newborn	11.3 ± .4	14.2 ± .3	15.4 ± .3

Table 11. Ratio of the treated body weight means to control body weight means - females

		Treated/Control					
Dose	Embryological age	Age in days					
		Birth	12	26	40	60	75
80r	10-1/2 days	.92	1.08	1.13	.99	.92	.93
160r	17-1/2 days	.98	.98	1.05	.91	.89	.89
160r	Newborn	1.03	.91	.86	.87	.84	.86
160r	14-1/2 days	.87	.90	.79	.75	.78	.79
320r	17-1/2 days	.95	.80	.80	.66	.72	.74
320r	Newborn	1.02	.81	.68	.68	.71	.74
320r	14-1/2 days	.73	1.12	.85	.56	.53	.57

the ratios closely approximate those of the males.

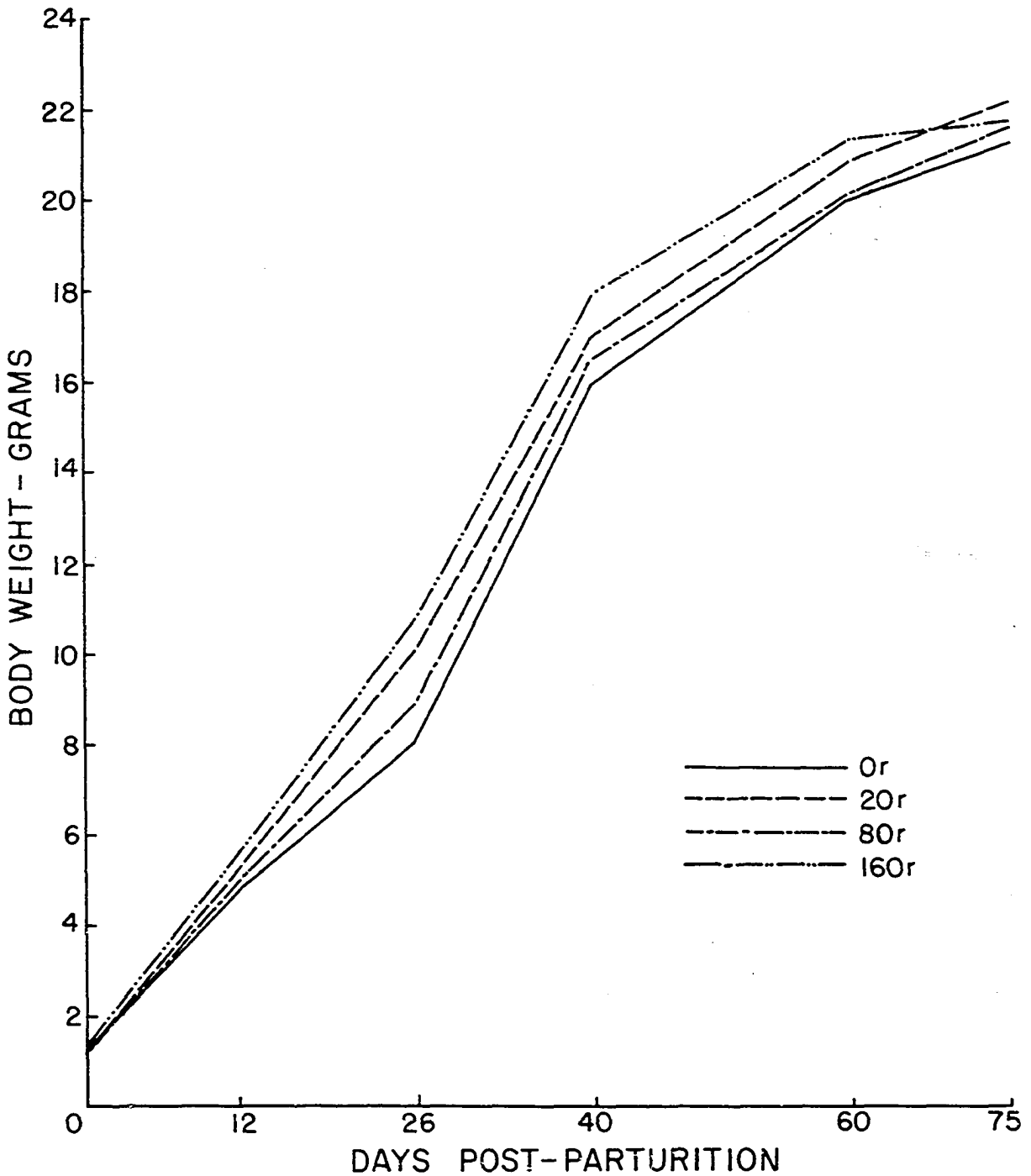
Influence of level of irradiation within each embryological age

Irradiation at 6-1/2 days (Figure 10) Irradiation at 6-1/2 days produced no decrease in growth rate except in the birth weights of animals irradiated with 160r. By 12 days, however, surviving progeny in this group outweighed the controls. The weight advantage disappeared by 60 days in contrast to the males where it persisted throughout the entire period of observation.

Irradiation at 10-1/2 days (Figure 11) This embryological stage was extremely sensitive to irradiation, even a dose of 80 r producing significantly lower birth weights. Although progeny surviving 80r weighed as much as controls at 12 days, deleterious effects were evident, as in the males, by 60 days. Mice that had been irradiated with 20r were heavier than controls from 12 days to 40 days, but were not statistically different from controls by 60 days. It may be recalled that in the males progeny that received 20r maintained a growth advantage over controls from 12 days through 75 days.

Irradiation at 14-1/2 days (Figure 12) Following irradiation at 14-1/2 days with 160r or 320r birth weights were lowered 13 and 27 per cent respectively. By 12 days only 160r animals were significantly lower than controls. At 26

Figure 10. Irradiation at 6-1/2 days. Females - body weight means, adjusted for litter size at birth.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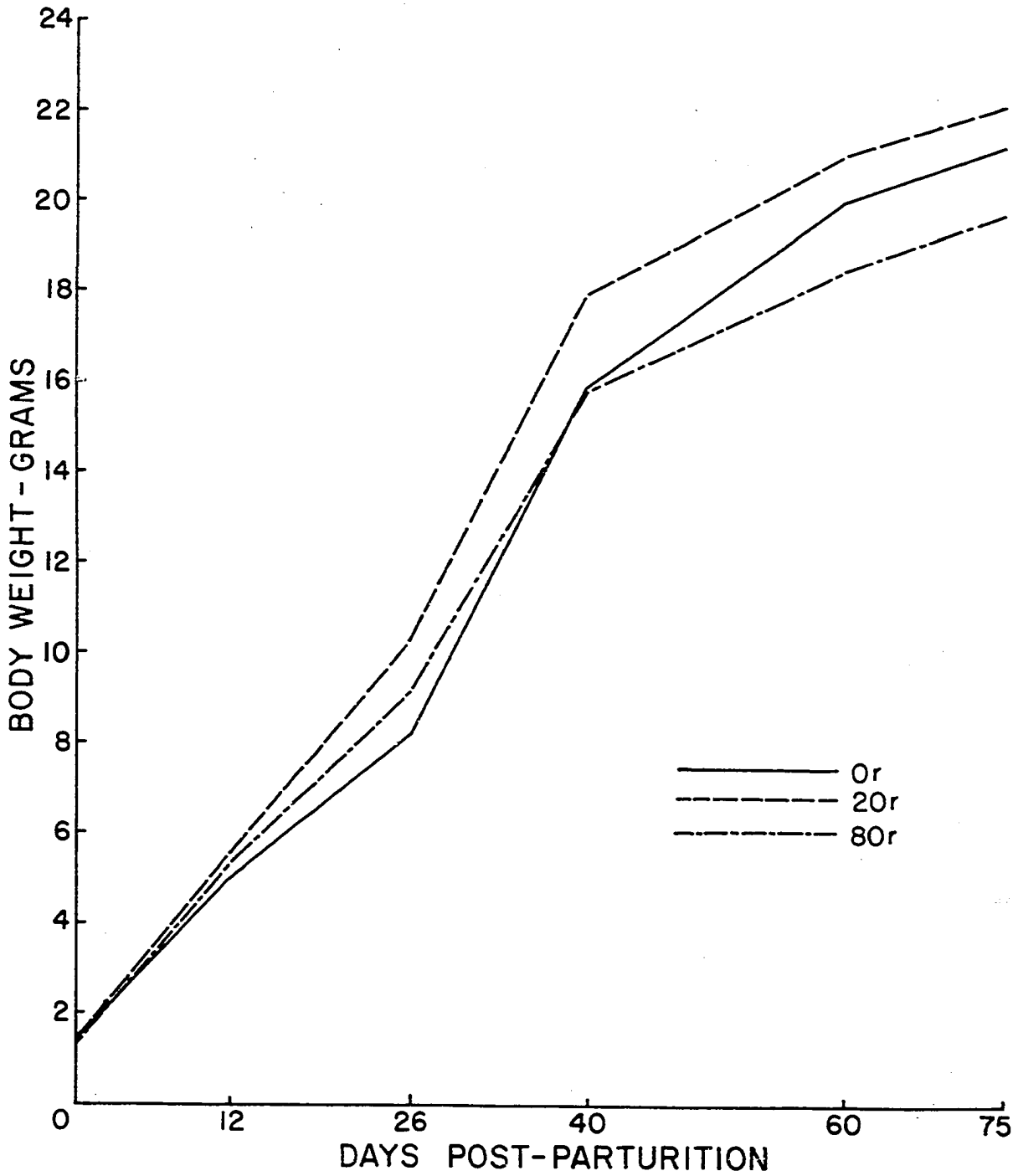
BODY WEIGHT - GRAMS
12
26
40
60
75
DAYS POST-PARTURITION
0r
20r
80r
160r

Figure 11. Irradiation at 10-1/2 days. Females - body weight means, adjusted for litter size at birth.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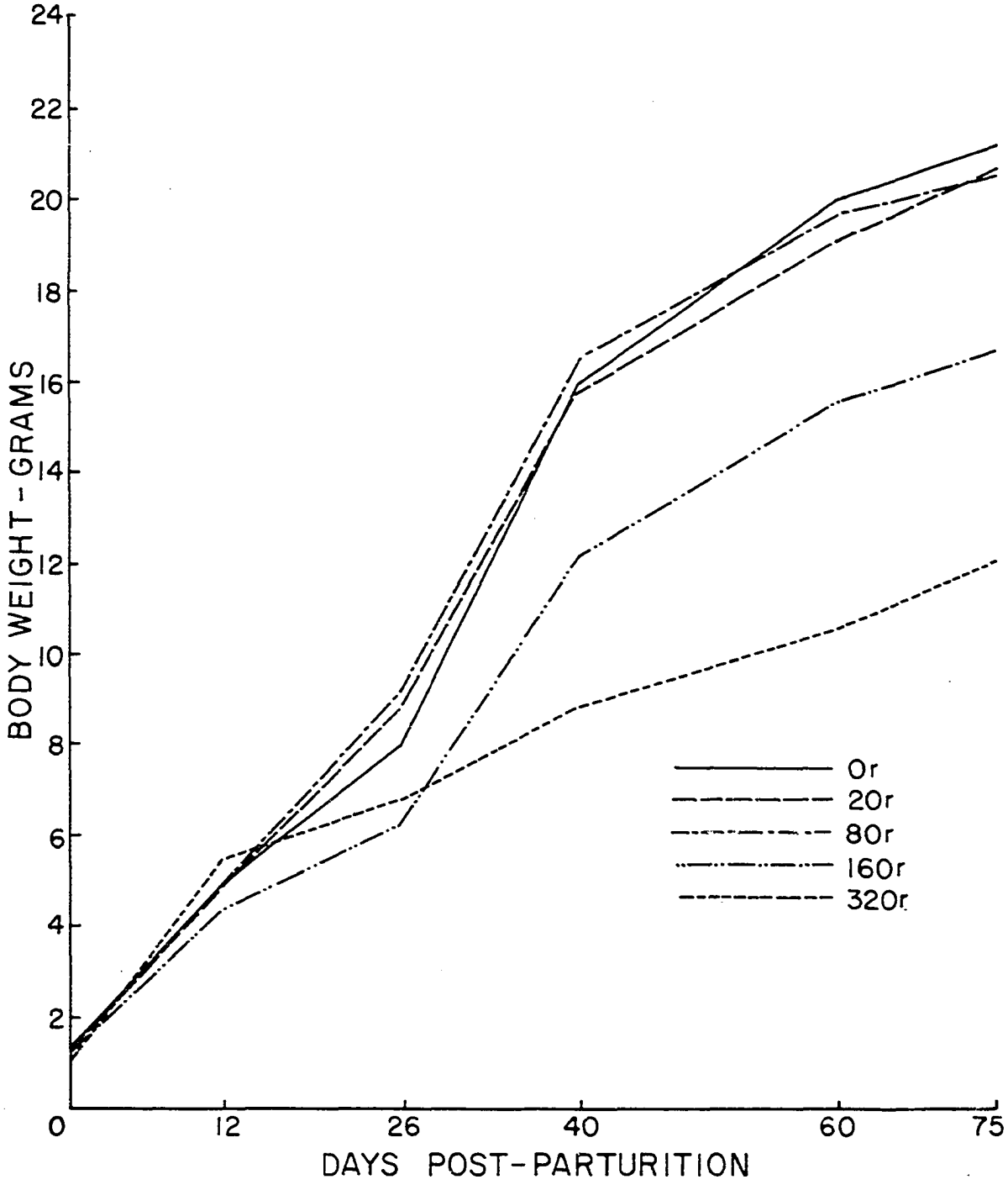
BODY WEIGHT - GRAMS
12
26
40
60
75
DAYS POST-PARTURITION
0r
20r
80r

Figure 12. Irradiation at 14-1/2 days. Females - body weight means, adjusted for litter size at birth.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BODY WEIGHT - GRAMS
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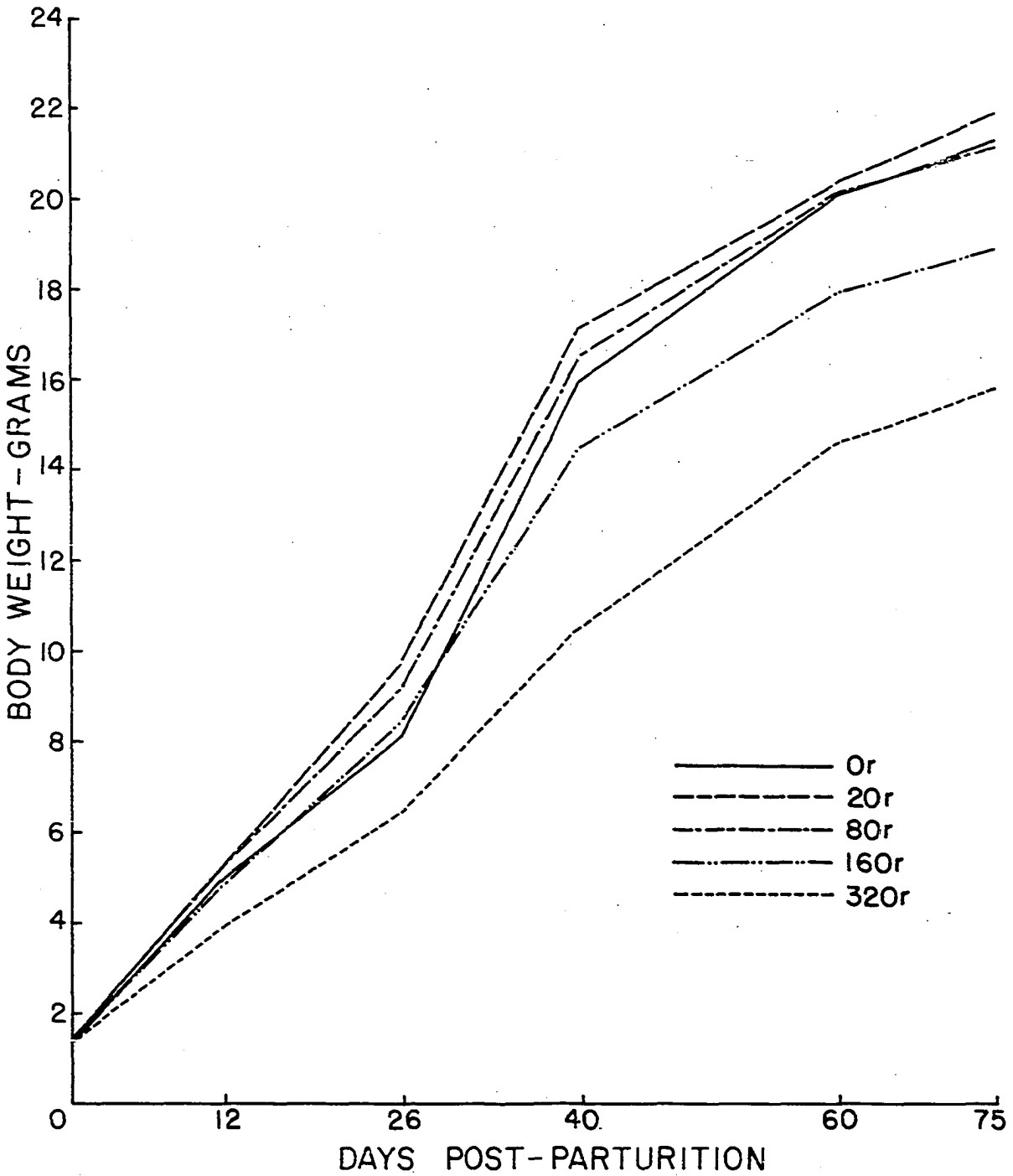
26
40
60
75
DAYS POST-PARTURITION
0r
20r
80r
160r
320r

days 320r animals were also lower than controls, but not significantly different from 160r progeny. By 40 days, however, progeny given 160r are significantly heavier than 320r progeny and maintain this advantage through 75 days. The relative magnitude of the difference between 160r and 320r animals is greatest at 60 days when 320r animals are only 68 per cent as heavy as 160r animals. Only one female given 320r survived the entire 75 day period, and weighed only 57 per cent of controls at 75 days.

Irradiation at 17-1/2 days (Figure 13) An effect on birth weight in the females is present after a dose of 320r. These progeny are always significantly lighter than controls, weighing only 68 per cent as much at 40 days. A significant difference in weight between 160r and controls is present by 60 days of age as in the males. Although animals given 20r and 80r consistently weigh more than controls, the differences are never statistically significant.

Irradiation at Term (Figure 14) A difference in body weights following irradiation of newborn animals is not achieved until 12 days with a dose of 320r. This dose causes a weight reduction of 32 per cent by 40 days and is still at 26 per cent by 75 days. By 40 days 160r progeny are also significantly different from controls as well as from 320r progeny. A significantly lower weight is observed at 60 days after 80r, but unlike in the males, the difference is no

Figure 13. Irradiation at 17-1/2 days. Females - body weight means, adjusted for litter size at birth.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BODY WEIGHT - GRAMS
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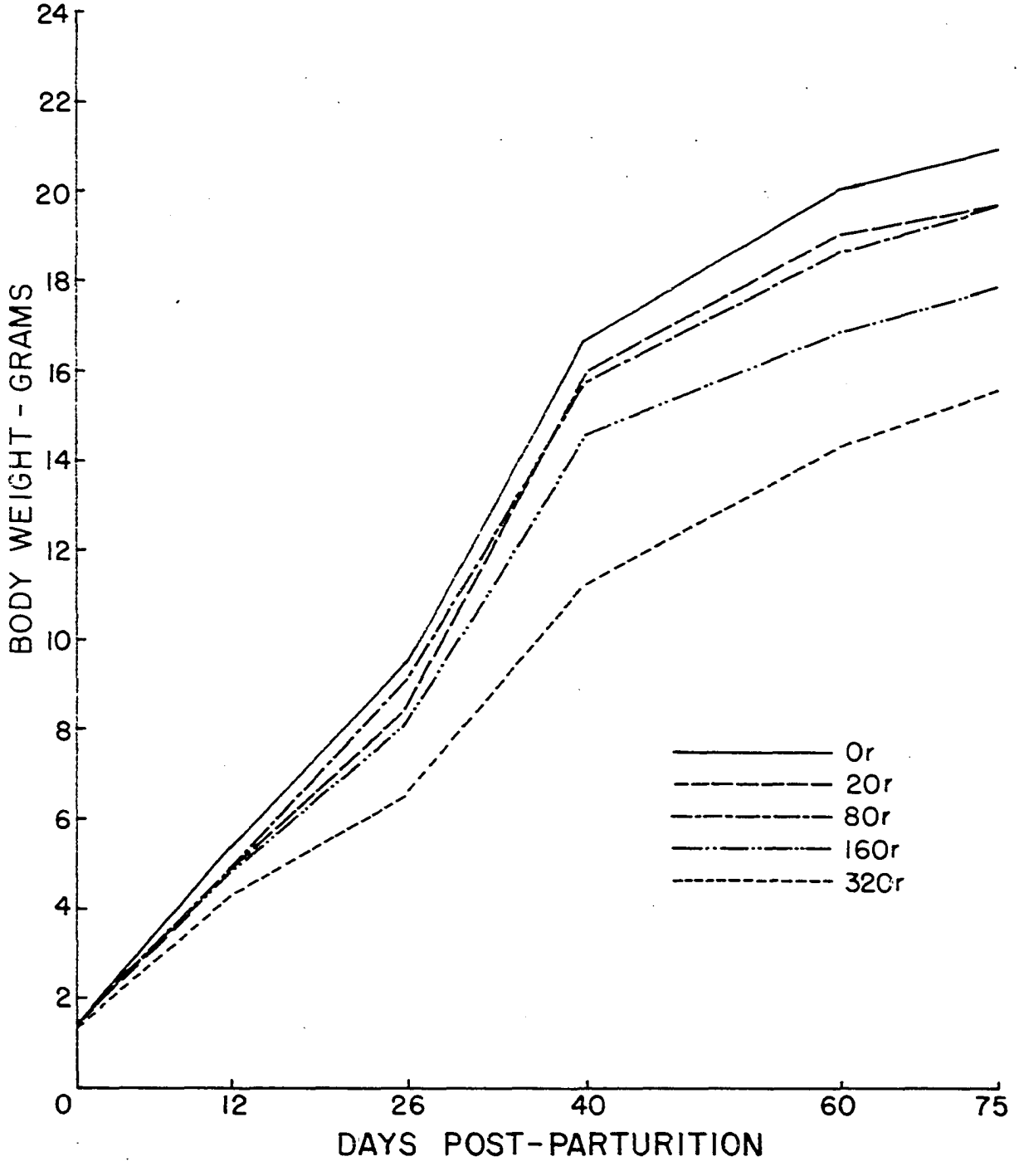
40
60
75
DAYS POST-PARTURITION
0r
20r
80r
160r
320r

Figure 14. Irradiation of newborn mice. Females - body weight means, adjusted for litter size at birth.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12
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40
60
75
DAYS POST-PARTURITION
0r
20r
80r
160r
320r

longer significant at 75 days.

Comparison of radiation response of males and females

A quantitative estimate of the effects of sex on the variations in body weights will be made in the section on components of variation. A general approximation of the effects of sex may be accomplished by examining the differences between male and female body weight means for each of the treatments. This difference has been expressed as the ratio of the female mean weights to the male mean weights. The results are included in Table 12. It is obvious that, for the most part, sex differences are constant over all treatments for single weight observations. Males are slightly heavier than females at birth, but there is virtually no difference between sexes by 12 days. Beginning at 26 days a small sex difference develops which continues through 75 days. The males are 16 per cent heavier on the average by 60 days and 17 per cent heavier by 75 days. The major exception to these generalities occurs in those progeny that were given 320r at 14-1/2 days. In these progeny the females are always heavier than males, from 12 days on, even at 75 days still being 32 per cent heavier. Mortality was extensive from this treatment, less than 10 per cent of the animals surviving to 75 days. The actual differences between the sex means in this treatment are not significant at any period due to the large standard error of the means.

Table 12. Ratio of female mean body weights to male mean body weights

Dose	Embryological age	Birth	Days post-parturition 12	26	40	60	75
0r		.94	.98	.90	.90	.86	.85
0r*		.96	1.00	1.00	.93	.84	.82
20r	6-1/2 days	.94	1.02	1.03	.88	.87	.85
	10-1/2 days	.96	1.00	.94	.89	.95	.85
	14-1/2 days	.96	.98	.94	.84	.81	.83
	17-1/2 days	.98	1.02	1.05	.95	.85	.86
	Newborn	.96	1.02	.93	.89	.84	.81
80r	6-1/2 days	.99	.93	.87	.85	.83	.83
	10-1/2 days	.97	1.04	.99	.87	.85	.85
	14-1/2 days	1.00	1.00	1.03	.95	.88	.87
	17-1/2 days	.97	1.04	1.00	.94	.86	.85
	Newborn	.98	.92	.96	.88	.83	.83
160r	6-1/2 days	.91	.95	.96	.89	.86	.81
	10-1/2 days	1.00	---	---	---	---	---
	14-1/2 days	.92	.90	.86	.83	.79	.78
	17-1/2 days	.98	1.02	1.00	.90	.85	.85
	Newborn	.99	1.02	.99	.95	.84	.82
320r	10-1/2 days	.93	---	---	---	---	---
	14-1/2 days	.98	1.38	1.17	1.17	1.19	1.32
	17-1/2 days	.95	.95	.97	.86	.84	.84
	Newborn	.96	1.02	.98	.91	.85	.82

*Represents the group of controls used in that part of the experiment dealing with irradiation of newborn animals.

Comparison of radiation response of inbreds and hybrids

Before examining the quantitative effects of genotype on variation in body weight response, it was felt desirable to investigate possible differential response to treatments of the two broad sub-populations of mice used in this study, the

inbreds and hybrids. For this purpose the ratio of

$$\frac{\text{Mean Body Weight Inbreds}}{\text{Mean Body Weight Hybrids}}$$

has been calculated for each observation period within each of the treatments. If a treatment did not have a differential effect of the two general types of progeny, the ratio obtained for each treatment should be comparable to that of the controls. The results are shown in Table 13.

It is obvious that there is considerable variation in this ratio over different treatments. In general, most of the values are less than one indicating an overall superiority of the hybrids. The minimum value of the ratio in the majority of cases occurred around 26 or 40 days. Thereafter in the period up to 75 days there was a general increase in ratios. At 75 days the hybrids were only slightly superior to the inbreds, the ratio at that time closely approximating the ratio that was found in the birth weights.

The difference between the inbreds and hybrids in each treatment appears comparable to the difference in the controls, although there are some evident exceptions. The exceptions, however, do not appear to follow any general trend either as to level of irradiation or embryological age, and it is probable the variations are just random fluctuations. These results indicate that the various treatments do not greatly alter the differences which are already present in these two classes of progeny.

Table 13. Ratio of inbred mean body weights to hybrid mean body weights

Embryological age	Dose	Days post-parturition: Birth	12	26	40	60	75
	0r	.95	.88	.61	.79	.90	.94
6-1/2 days	20r	.86	1.02	.93	.95	.99	1.02
	80r	.98	1.04	1.10	1.01	1.03	1.03
	160r	.98	.86	.86	.92	.92	.95
10-1/2 days	20r	.92	.89	.81	.88	.94	.94
	80r	.98	.98	.86	.92	.98	.99
	160r	1.01	--	--	--	--	--
	320r	1.12	--	--	--	--	--
14-1/2 days	20r	.98	1.00	.86	.89	.97	1.00
	80r	.96	1.02	.87	.86	.91	.91
	160r	1.03	1.07	1.11	1.08	.99	1.02
	320r	.91	--	--	--	--	--
17-1/2 days	20r	.96	.82	.77	.81	.92	.95
	80r	.96	.96	.74	.83	.94	.94
	160r	.94	.98	.82	.81	.93	.95
	320r	.91	.80	.65	.67	.79	.83
Newborn	0r	1.04	1.00	.79	.79	.93	.92
	20r	.96	1.17	.80	.91	.98	.96
	80r	.96	.94	.77	.84	.91	.94
	160r	.96	.84	.84	.78	.81	.85
	320r	.94	1.05	.80	.86	.88	.92

Estimation of components of variation

The amount of variation in body weight response to *in utero* irradiation can be partitioned additively into the amounts due to the various effects and their interactions by utilizing the estimated components of variance derived

from an analysis of variance. Within each embryological age the experimental design is essentially a factorial type. There are 9 genotypes or hereditary types, and, depending on the embryological age, from 3 to 5 dosage levels, and from 27 to 45 heredity by dosage cells. The interaction indicates the amount of variation that is attributable to the differential responses of the different genotypes to irradiation.

The general mathematical model upon which the component analysis is based is:

$$Y_{ijkl} = u + g_i + t_j + (gt)_{ij} + f_k + (fg)_{ik} + (ft)_{jk} + (fgt)_{ijk} + e_{ijkl}$$

where u = the overall mean

i = 1, 2, ..., 9 - the hereditary types

j = 1, 2, 3 (or 4 or 5) - the dosage levels

k = 1, 2 - the sexes

As there were disproportionate sub-class numbers within each embryological age, the method of unweighted means was used in the analysis of variance. Although this method is approximate, it was felt that since the sub-class numbers were only slightly unequal, the approximation would be close enough so that the general features of the experiment could be readily interpreted.

The general breakdown for the analysis is given in Table 14.

The components can be interpreted as follows: G is the variation due to genotypic or hereditary differences, T is the

Table 14. Breakdown for the statistical analysis

Source of variation	d.f.	Components of variation
Between genotypes	8	E + 2j G
Between dosages	(j-1)*	E + 18 T
Genotype x dosage	8(j-1)	E + 2 GT
Between sexes	1	E + 9j F
Sex x genotype	8	E + j FG
Sex x dosage	(j-1)	E + 9 FT
Sex x genotype x dosage	8(j-1)	E + FGT
Error		E

*j = 3, 4 or 5 depending on which embryological age was analyzed.

variation due to differences in effects of the dosage levels, and F is the variation between sexes. The interaction terms are interpreted as arising from the differential responses of the genotypes or sexes from one level of irradiation to the next. The term, E, is considered due to uncontrollable environmental variation, and represents random variation of individual differences of mice of the same sex within a litter that were given the same treatment. The term that was used as an estimate of random variation within the analysis of each embryological age was obtained by dividing the mean square of individuals within sub-classes by the reciprocal of the mean of the reciprocals of the sub-class numbers. This value was determined for all embryological ages combined, and used as the estimate of unaccounted for variation in the analysis of each embryological age.

Irradiation at 6-1/2 days The results of the component analysis are presented in Table 15 and Figure 15. The magnitude of the dose effect, which is only 2.6 per cent at 75 days, is further evidence that mice of this embryological age are little affected by irradiation as far as weight response is concerned. The dose effect does reach a maximum of 22.0 per cent at 26 days after birth but declines thereafter. The decline in dose effect does not represent a recovery response to the effects of irradiation in so much as it was the controls that grew relatively slowly up to the time of weaning.

Genotypic differences in body weight are irregular, declining to only 4.2 per cent by 75 days from an initial percentage at birth of 23.1. The effect remains more or less the same through 40 days and then declines rapidly.

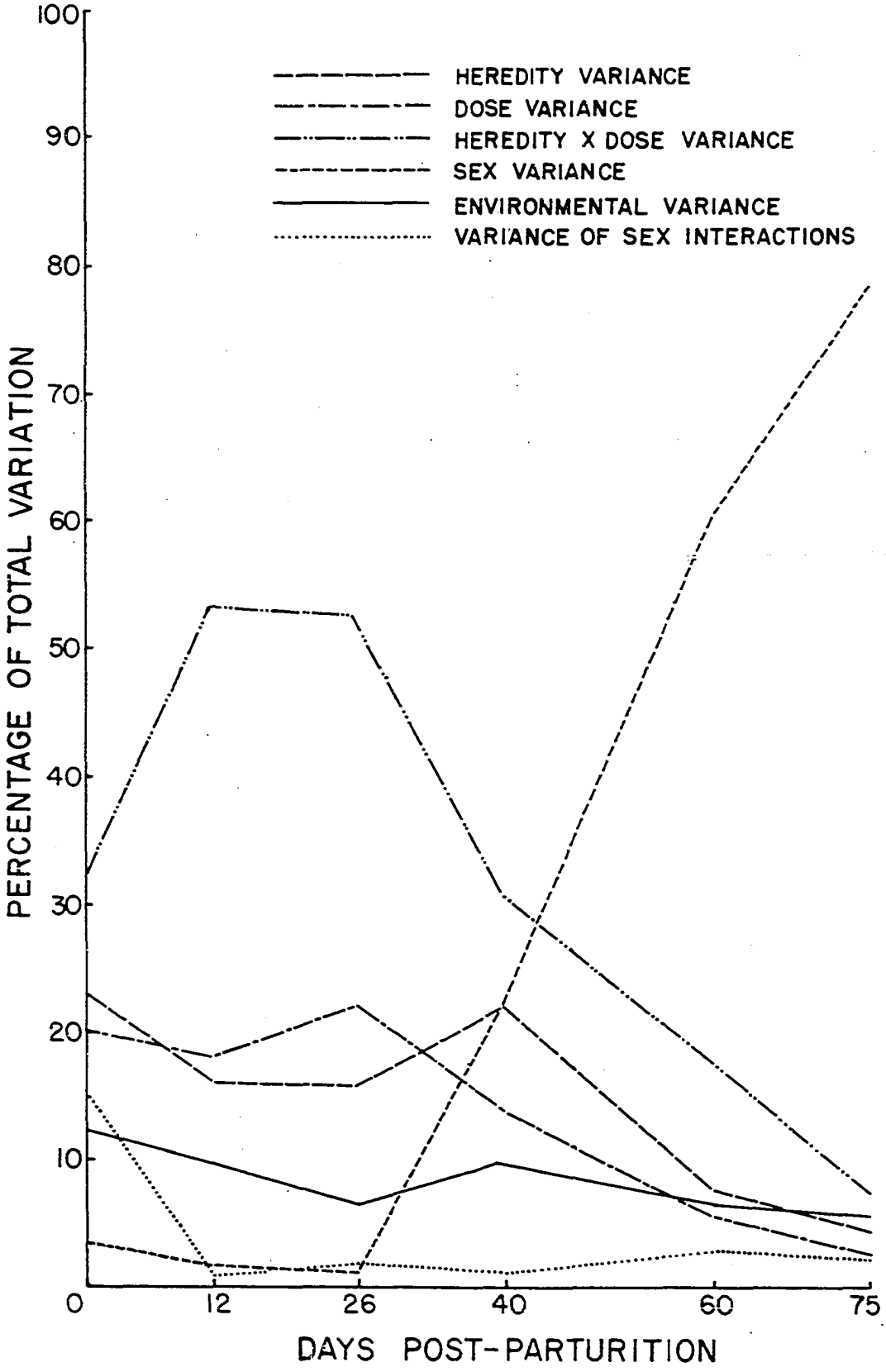
The heredity by dose component, which indicates the differential response of various genotypes to irradiation, reaches a maximum 12 days after birth when 53.3 per cent of the total variance is due to these genetic differences in body weight response. This component declines rapidly after 26 days and accounts for only 7.3 per cent of the total variation at 75 days.

The sex difference in weight, which was 15.0 per cent at birth decreases to a negligible amount through 26 days and then progressively increases to a high of 78.2 per cent at 75.

Table 15. Irradiation at 6-1/2 days - breakdown of variation in body weight into the components: percentage of total variation

Component of variation	Age in days post-parturition					
	Birth	12	26	40	60	75
Heredity effect	23.1	16.2	15.8	21.8	7.5	4.2
Dose effect	3.4	18.1	22.0	13.7	5.4	2.6
Heredity x dose effect	32.1	53.3	52.6	30.8	17.5	7.3
Sex effect	15.0	1.7	1.1	22.8	60.2	78.2
Sex x heredity effect	7.2	1.2	1.4	0.9	3.0	1.2
Sex x dose effect	6.0	0	0.5	0.4	0	1.0
Sex x dose x heredity effect	0	0	0	0	0	0.1
Error	12.3	9.5	6.6	9.6	6.4	5.4

Figure 15. Irradiation at 6-1/2 days. Breakdown of variation in body weight. Components expressed as a percentage of total variation.


HEREDITY VARIANCE
DOSE VARIANCE
HEREDITY X DOSE VARIANCE
SEX VARIANCE
ENVIRONMENTAL VARIANCE
VARIANCE OF SEX INTERACTIONS
PERCENTAGE OF TOTAL VARIATION
100
90
80
70
60
50
40
30
20
10
0
12
26
40
60
75
DAYS POST-PARTURITION

The sex difference in weight, which was 15.0 per cent at birth decreases to a negligible amount through 26 days and then progressively increases to a high of 78.2 per cent at 75 days. The various effects involving the interaction of sex, such as sex by heredity, sex by dose and sex by dose by heredity, are for the most part quite small indicating that the differences between the sexes are not affected either by level of irradiation or by genotype.

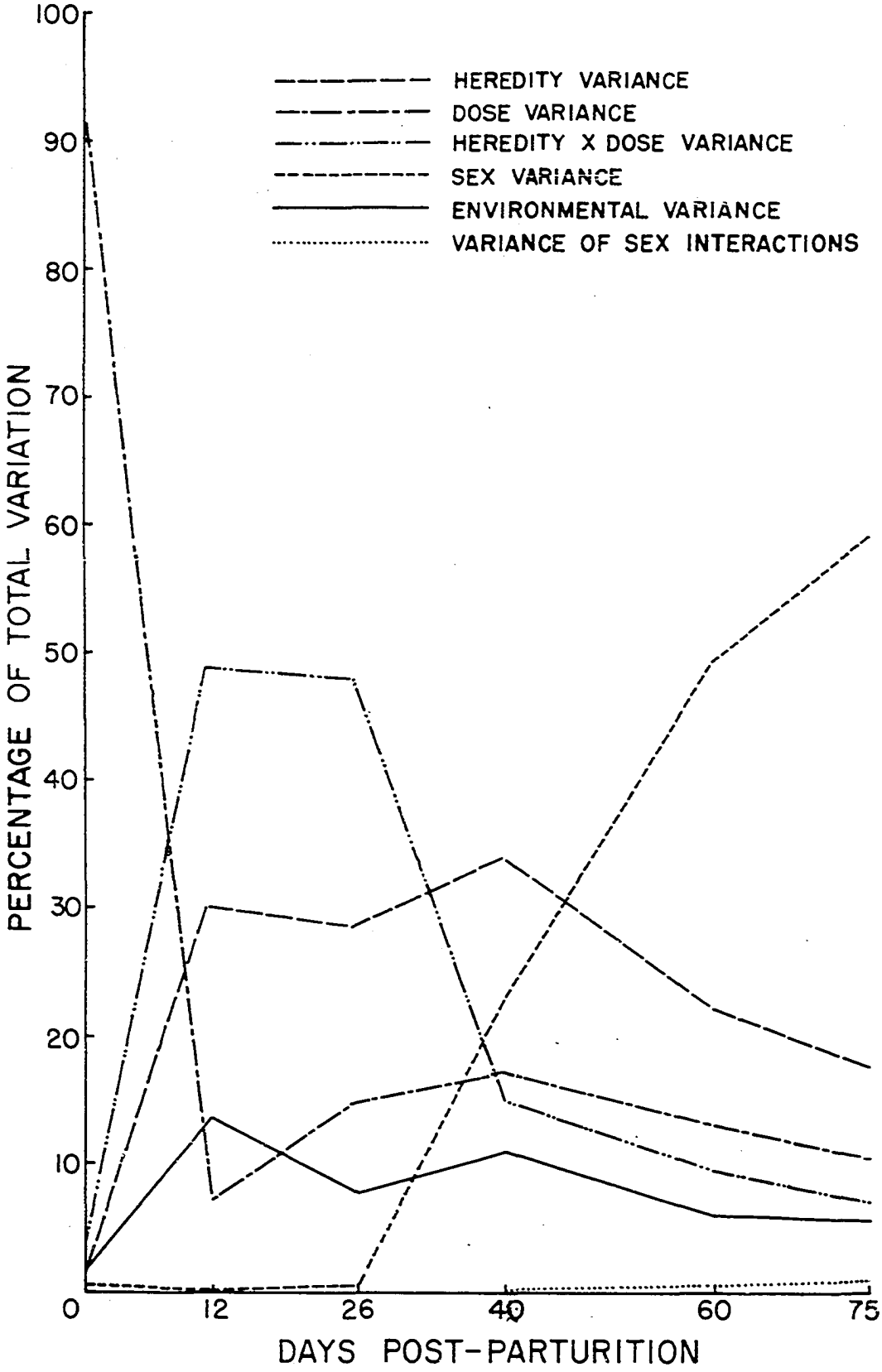
Uncontrollable environmental variation, from a maximum of 12.3 per cent at birth drops gradually to a minimum of 5.4 per cent by 75 days.

Irradiation at 10-1/2 days The results of the component analysis of irradiation at 10-1/2 days are given in Table 16 and Figure 16. As seen in the table there are two distinct phases of response. For the birth weight most of the total variation, some 90.6 per cent, is due to the dose effect. It was seen earlier that this age was the most sensitive to birth weight changes, body weights averaging less than half of controls after a dose of 320r. The tremendous effect of dose at this time tends to mask the other possible causes for variation. By 12 days of age the picture changes considerably, largely due to the fact that the more severely stunted progeny that had been exposed to 160r or 320r suffer a 100 per cent mortality. The dose effect at 12 days is only 7.1 per cent and after a high of 17.3 per cent at 40 days

Table 16. Irradiation at 10-1/2 days - breakdown of variation in body weight into the components: percentage of total variation

Component of variation	Age in days post-parturition					
	Birth	12	26	40	60	75
Heredity effect	0.9	30.2	28.6	33.9	22.1	17.4
Dose effect	90.6	7.1	15.1	17.3	12.9	10.3
Heredity x dose effect	6.1	49.0	48.1	14.9	9.6	6.9
Sex effect	0.6	0	0.4	22.8	49.1	58.9
Sex x heredity effect	0	0	0	0	0	0.9
Sex x dose effect	0.1	0	0	0	0	0
Sex x dose x heredity effect	0	0	0	0	0.4	0
Error	1.7	13.7	7.8	11.1	5.9	5.6

Figure 16. Irradiation at 10-1/2 days. Breakdown of variation in body weights. Components expressed as a percentage of total variation.


HEREDITY VARIANCE
DOSE VARIANCE
HEREDITY X DOSE VARIANCE
SEX VARIANCE
ENVIRONMENTAL VARIANCE
VARIANCE OF SEX INTERACTIONS
PERCENTAGE OF TOTAL VARIATION
100
90
80
70
60
50
40
30
20
10
0
12
26
40
60
75
DAYS POST-PARTURITION

declines to 10.3 per cent at 75 days, again indicating that the dose effect at 75 days in surviving progeny is relatively small.

The differences in genotypes rise abruptly to 30.2 per cent at 12 days, and after a high of 33.9 per cent at 40 days declines to 17.4 per cent at 75 days.

The heredity by dose effect follows the same pattern as that after irradiation at 6-1/2 days. The maximum effects of 49.0 per cent occurs at 12 days, but by 75 days this interaction accounts for only 6.9 per cent of the total variation.

Sex differences are negligible until 40 days when the mean weights diverge considerably. At 75 days the sex effect accounts for 58.9 per cent of the total variation. The different interactions of sex are always small.

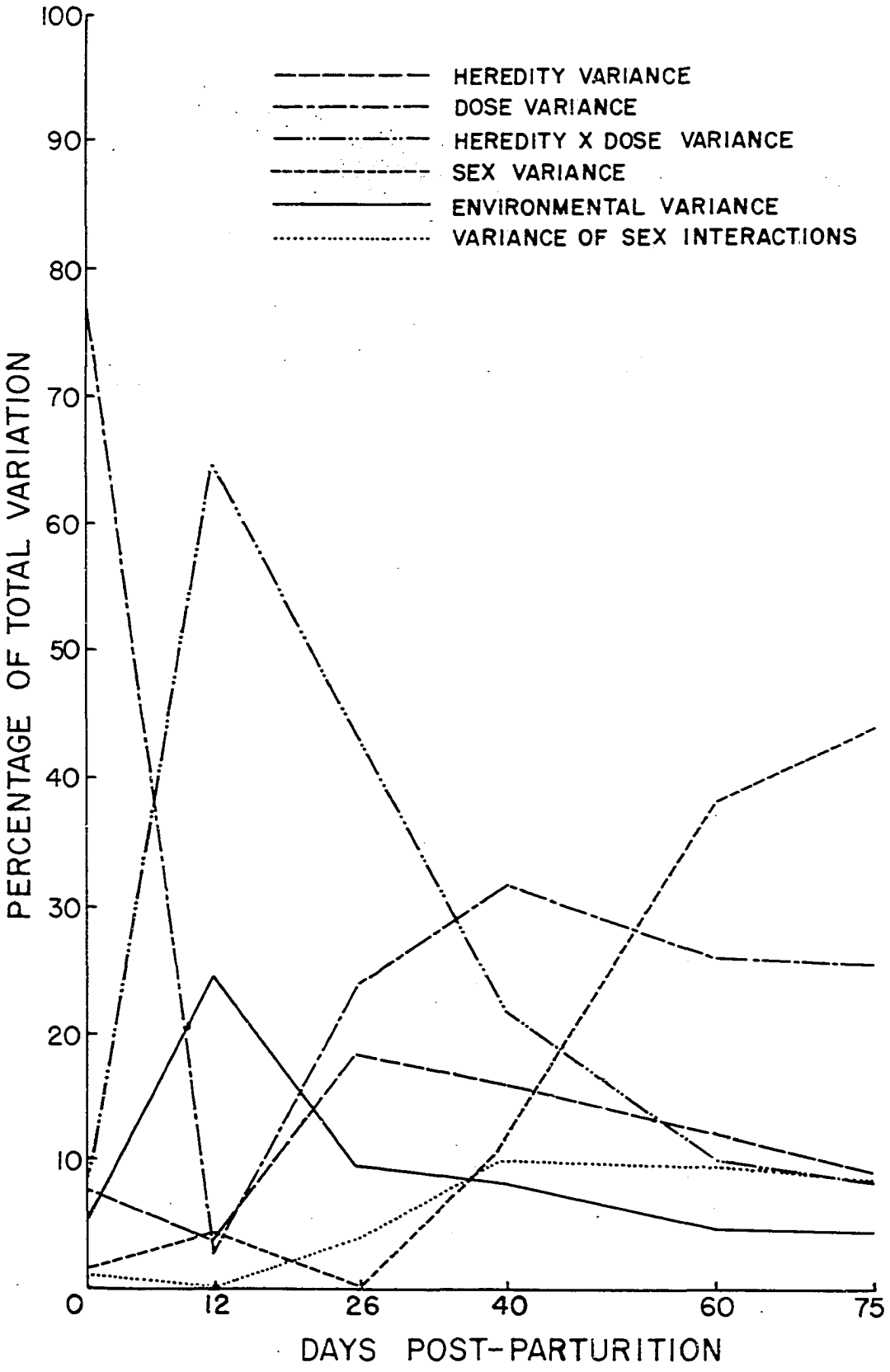
Random variation effects are quite similar to those at 6-1/2 days. The maximum effect is reached at 12 days when 13.7 per cent of the variation is due to this cause. By 75 days uncontrollable variation accounts for only 5.6 per cent of the total variation.

Irradiation at 14-1/2 days The component analysis of irradiation at 14-1/2 days, presented in Table 17 and Figure 17, also has two distinct phases of response. The subdivision of the variation of birth weights is considerably different from the subdivision of the later weights. This embryological age was second to 10-1/2 days in sensitivity to irradiation

Table 17. Irradiation at 14-1/2 days - breakdown of variation in body weight into the components: percentage of total variation

Component of variation	Age in days post-parturition					
	Birth	12	26	40	60	75
Heredity effect	7.7	3.5	18.3	16.1	12.1	9.2
Dose effect	76.9	2.7	23.8	31.7	25.8	25.4
Heredity x dose effect	7.4	64.7	43.8	21.9	10.0	8.4
Sex effect	1.5	4.4	0.3	12.0	38.0	44.0
Sex x heredity effect	0	0	0	1.5	3.2	2.8
Sex x dose effect	1.0	0	0.1	1.7	1.7	1.5
Sex x dose x heredity effect	0	0	3.9	7.0	4.7	4.3
Error	5.5	24.7	9.8	8.1	4.5	4.4

Figure 17. Irradiation at 14-1/2 days. Breakdown of variation in body weight. Components expressed as a percentage of total variation.


HEREDITY VARIANCE
DOSE VARIANCE
HEREDITY X DOSE VARIANCE
SEX VARIANCE
ENVIRONMENTAL VARIANCE
VARIANCE OF SEX INTERACTIONS
PERCENTAGE OF TOTAL VARIATION
100
90
80
70
60
50
40
30
20
10
0
12
26
40
60
75
DAYS POST-PARTURITION

effects at birth. This is reflected in the large percentage, 76.9, of the total variation that is attributable to dose effects. The smallest progeny, which resulted from a dose of 320r, weighed only about 70 per cent of the controls at birth. Almost all of these progeny died within a few days after birth. At 12 days then the dose effect accounts for only 2.7 per cent of the variation in body weight. By 26 days this effect rises abruptly to 23.8 per cent, reaches a maximum of 31.7 per cent at 40 days, and then appears to be leveling off at 75 days, when a little over a fourth of the total variation is due to the effects from the different levels of irradiation.

Hereditary differences which make up 7.7 per cent of the total variation in birth weights decline to 3.5 per cent at 12 days, and then reach their maximum of 18.3 per cent at 26 days. In the succeeding period up to 75 days there is a slow progressive decline with hereditary differences comprising 9.2 per cent of the variation in weights at 75 days.

The interaction between genotypes and level of irradiation is 7.4 at birth, but rises abruptly to a maximum of 64.7 per cent at 12 days. By 40 days this effect is reduced to one-third of this value, and it continues to decline to 75 days, when it contributes only 8.4 per cent of the total variation.

Differences between sexes are quite small until 40 days when an abrupt increase commences that reaches a maximum of

44.0 per cent by 75 days. The various effects involving interactions with sex are always small and do not contribute significantly to the total variation in body weights.

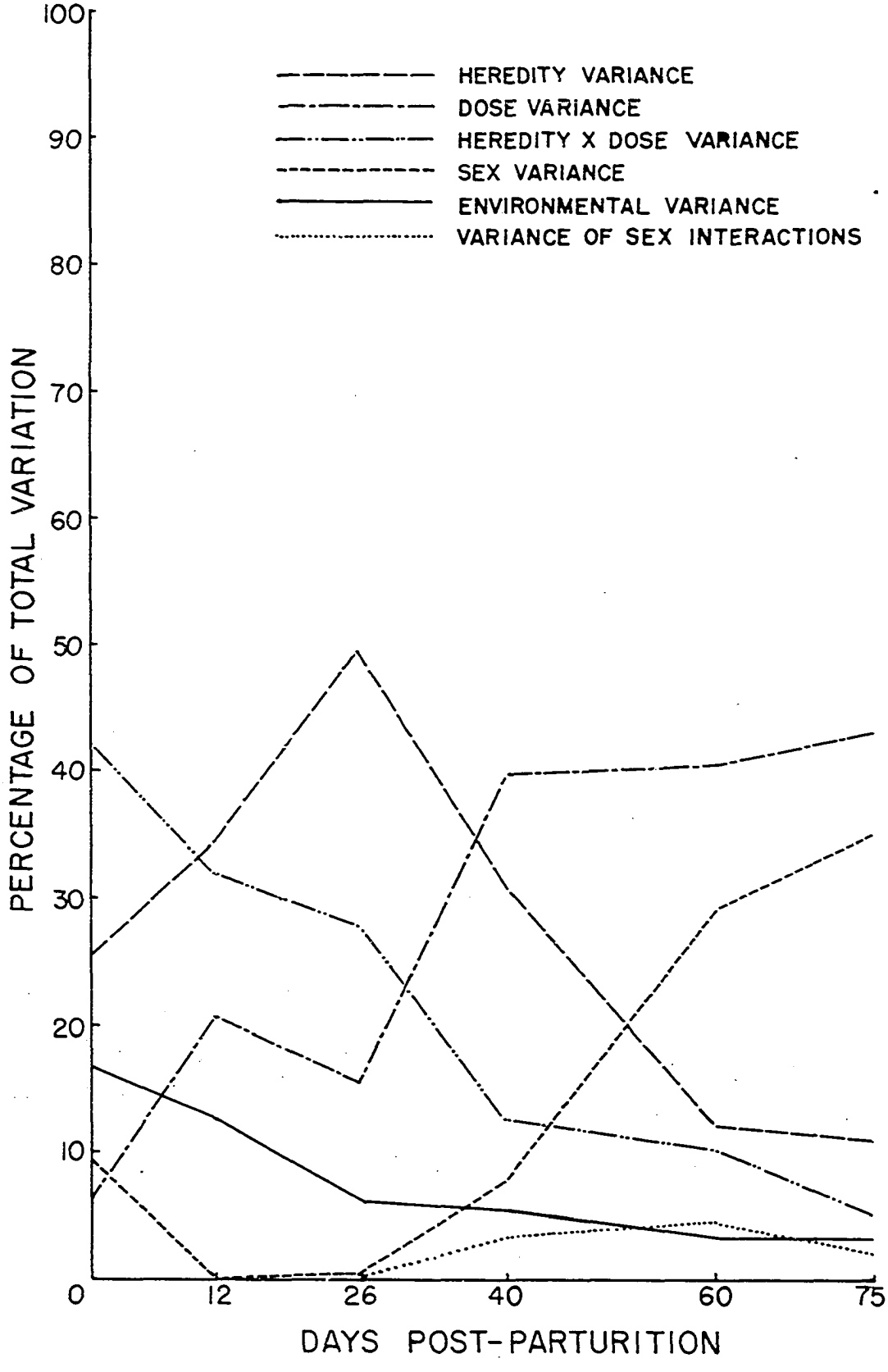
Environmental variations make up 5.5 per cent of the variation in birth weights. This effect reaches a maximum at 12 days when almost one-fourth of the total variation is due to this random source. The effect declines in importance after that, and reaches its minimum of 4.4 per cent at 75 days.

Irradiation at 17-1/2 days The data from this component analysis are presented in Table 18 and Figure 18. Irradiation at this stage, relatively late in gestation, leaves little time for the effects of irradiation to take place in the birth weights. This is observed in the low contribution to total variation of birth weights by the dose effect, which is only 5.9 per cent. There are some survivors after all levels of irradiation at this age, so that even progeny that received 320r contribute to the variation at ages after birth. Thus, by 12 days the dose effect rises to 20.6 per cent, which is the highest of any of the embryological ages at 12 days. It declines slightly at 26 days but then rises sharply to 39.9 per cent and further increases to 43.2 per cent of the total variation at 75 days. Of the embryological ages used in this study the magnitude of the dose effect at 75 days is by far the largest after irradiation

Table 18. Irradiation at 17-1/2 days - breakdown of variation in body weight into the components: percentage of total variation

Component of variation	Age in days post-parturition					
	Birth	12	26	40	60	75
Heredity effect	25.6	34.3	49.9	31.0	12.2	11.1
Dose effect	5.9	20.6	15.6	39.9	40.5	43.2
Heredity x dose effect	42.4	32.1	27.8	12.4	10.1	5.1
Sex effect	9.5	0.2	0.4	7.8	29.3	35.0
Sex x heredity effect	0	0	0.2	2.0	0.5	0
Sex x dose effect	0	0	0	0	1.7	1.1
Sex x dose x heredity effect	0	0	0	1.5	2.3	1.1
Error	16.6	12.8	6.1	5.4	3.4	3.4

Figure 18. Irradiation at 17-1/2 days. Breakdown of variation in body weight. Components expressed as a percentage of total variation.


HEREDITY VARIANCE
DOSE VARIANCE
HEREDITY X DOSE VARIANCE
SEX VARIANCE
ENVIRONMENTAL VARIANCE
VARIANCE OF SEX INTERACTIONS
PERCENTAGE OF TOTAL VARIATION
100
90
80
70
60
50
40
30
20
10
0
12
26
40
60
75
DAYS POST-PARTURITION

at 17-1/2 days.

Differences in the response of the genotypes even at birth accounts for 25.6 per cent of the total variation. The genotypic effect increases to a maximum of 49.9 per cent at 26 days. This percentage is the largest of any of the heredity effects at any embryological age. The hereditary differences decline progressively after 26 days, but still represent 11.1 per cent of the variation in weights at 75 days.

Although the dose effect was only 5.9 per cent at birth, there was considerable differential response to irradiation between the different genotypes as is evidenced by the heredity by dose effect, which is 42.4 per cent at birth. This interaction declines gradually after birth and at 75 days, although there is considerable response to the different levels of irradiation, there is little difference in the response of the various genotypes as seen by the small contribution of this interaction which is only 5.1 per cent.

The sex differences follow the same general pattern as is found in the other embryological ages. There is a small amount of variation at birth, this effect contributing 9.5 per cent of the variation, but sex differences are almost nil at both 12 days and 26 days. Differences become obvious again at 40 days and then increase sharply by 75 days, when the sex effect is responsible for slightly over one-third of the total variation. The interactions with sex are always

small.

Environmental variation reaches its maximum at birth when 16.6 per cent of the total variation is due to this source. However, this effect declines steadily after that and represents only 3.4 per cent of the variation in weight at 75 days.

Irradiation at birth The results of the component analysis of irradiation of newborn animals are shown in Table 19 and Figure 19. The components of variance were not calculated for the birth weights since the mice were actually weighed before treatment. By 12 days differences due to the effects of the different levels of irradiation have become apparent as seen in the 10.8 per cent contribution to body weight variation. The dose effect reaches its maximum of 41.1 per cent at 40 days and then declines slightly by 75 days when it constitutes slightly less than a third of the total variation.

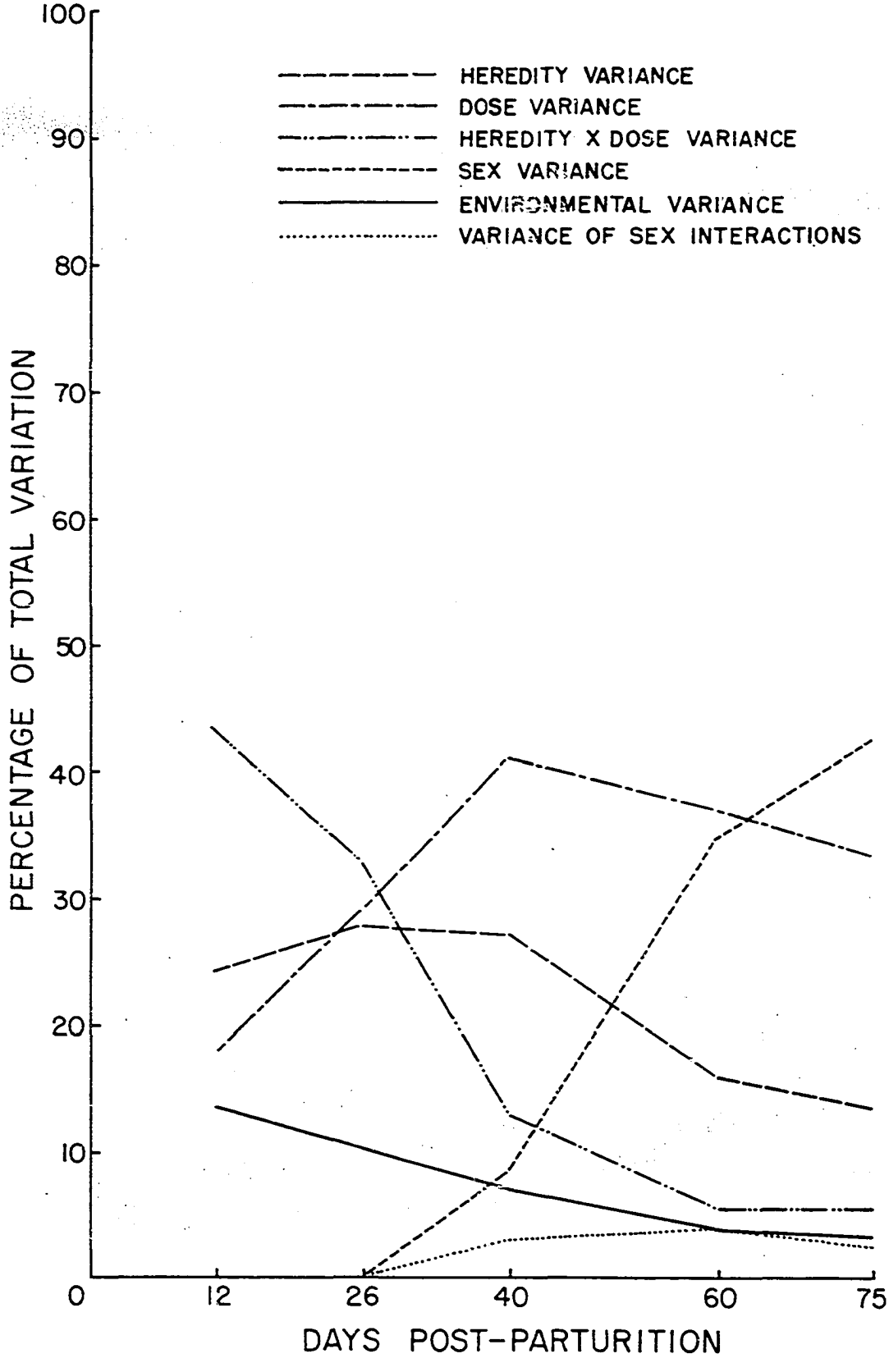
Genotypic differences, which account for 24.3 per cent of the total variation at 12 days, increase to a high of 27.7 per cent at 26 days and then decline gradually to 75 days. At 75 days genotypic variation accounts for 13.4 per cent of the variation. The magnitude of this difference closely approximates that found following irradiation at 17-1/2 days.

The heredity by dose interaction is at its maximum of 43.5 per cent at 12 days. At that time, it has the most effect of any of the factors contributing to variation in body

Table 19. Irradiation of newborn animals - breakdown of variation in body weight into the components: percentage of total variation

Component of variation	Age in days post-parturition					
	Birth	12	26	40	60	75
Heredity effect	10.8	24.3	27.7	27.2	15.8	13.4
Dose effect	0.3	18.0	29.0	41.1	36.7	33.0
Heredity x dose effect	51.6	43.5	32.8	13.1	5.3	5.4
Sex effect	6.8	0	0.1	8.5	34.5	42.4
Sex x heredity effect	0	0	0	0.3	0.8	0
Sex x dose effect	0	0.6	0	0.3	0.7	0.8
Sex x dose x heredity effect	0	0	0	2.3	2.5	1.6
Error	30.5	13.6	10.4	7.2	3.7	3.4

Figure 19. Irradiation of newborn animals. Breakdown of variation in body weight. Components expressed as a percentage of total variation.


HEREDITY VARIANCE
DOSE VARIANCE
HEREDITY X DOSE VARIANCE
SEX VARIANCE
ENVIRONMENTAL VARIANCE
VARIANCE OF SEX INTERACTIONS
PERCENTAGE OF TOTAL VARIATION
100
90
80
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20
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0
12
26
40
60
75
DAYS POST-PARTURITION

weight. It decreases slightly at 26 days, and then drops sharply so that by 75 days this effect can account for only 5.4 per cent of the total variation. The effect closely parallels that found after irradiation at 17-1/2 days in the respect that, although there is a large response to the various levels of irradiation, there is almost no differential response of the genotypes to these different doses.

There is no variation attributable to sex differences at 12 days and almost none at 26 days. Beginning at 40 days the sex effect begins to increase. It climbs sharply to 34.5 per cent at 60 days, and at 75 days comprises 42.4 per cent of the variation. The various interactions involving sex are small at all ages.

Random variation accounts for 13.6 per cent of the total variation at 12 days. There is a progressive decrease in the following ages, and at 75 days this effect accounts for only 3.4 per cent of the total variation.

General summary of the components of variance analysis over all embryological ages

Although there is considerable variation between the different embryological ages in the percentages of total variation that are attributable to the various factors operative in this experiment, there are some general patterns that hold true for all ages. Within these embryological ages where progeny show the greatest body weight response, not

including the birth weight response, to irradiation the effects of the irradiation do not reach their maximum contribution to the total variation usually until 40 days or more after birth. Furthermore, this effect declines little, if at all, by 75 days.

Slight genotypic differences are usually already present in the birth weights. They do not reach a maximum until 26 to 40 days. By 75 days the differences have diminished, and the percentage of total variation attributed to these differences closely approximates that found in the birth weights. It was seen earlier that the hybrid mice had an initial birth weight advantage over the inbreds, and that this advantage was increased through the time of weaning. After weaning the inbreds made up most of the weight difference, and by 75 days the relative difference between inbreds and hybrids was similar to that found in birth weights. This emphasizes that most of the variation attributed to genotypic differences is due to differences between the two broad subclasses of inbreds and hybrids, although there are differences between the various inbreds and hybrids.

The differential response of the genotypes to different levels of irradiation, as measured by the heredity by dose effect, usually reaches its maximum contribution to the total variation between 12 and 26 days, and then declines progressively so that by 75 days this effect contributes little to

the variation in body weight, even though there may be considerable variation due to the effects of the different levels of irradiation.

A definite sex effect exists at birth, but it has largely disappeared by 12 days. At 40 days the sex difference starts to increase and achieves its maximum at 75 days when an average of over 50 per cent of the total variation can be attributed to the sex differences in body weight. The interactions of sex with heredity, dose and heredity and dose jointly are always negligible.

Random variation is, in general, at its maximum at birth or 12 days and then declines to its minimum at 75 days when it comprises less than 5 per cent of the total variation.

DISCUSSION

In evaluating effects of *in utero* irradiation it is necessary to consider the possible role of the maternal organism in producing abnormal development in the embryo. It seems likely that the cases of death of whole litters are due not so much to the direct effect of radiation on the embryo as they are to injury of the maternal reproductive system. The data of Russell and Russell (1950a) show that the number of uteri with no implants following irradiation *in utero* is excessively large as compared with those having 1, 2, etc., implants indicating that the direct radiation death of every embryo in a litter cannot account for the entire effect. Since there is no evident correlation between the averages for total death and death due to loss of whole litters for irradiation on different embryological ages, it also appears that the direct radiation death of a number of embryos sufficiently large to exert an adverse effect of the viability of the remaining embryos in the uterus cannot account for much of the loss of entire litters. Brent (1957) in an experiment designed specifically to examine the indirect effects of irradiation on rat embryos concluded that the metabolic disturbances that are produced in the radiation syndrome in the maternal organism do not increase the resorption rate in most of the experimental animals but do cause 100 per cent resorption in some of the litters. Grayevsky, *et al.*

(1959) obtained completely viable offspring from rats which had been irradiated with doses of 400r and 600r. Irradiation of the dam apparently had an effect only in those cases in which the entire litter died. In these cases irradiation of the dam with 400r and 600r and the embryos with 200r had an effect that was similar to the direct irradiation of the embryos with 300r. It can be concluded from these various studies that the loss of entire litters after in utero irradiation is the result mostly of the radiation effect on the mother which prevents continuation of the pregnancy.

It would seem likely from these results that in the present experiment the failure of any litters to have been born following irradiation with 320r at 6-1/2 days, and the decrease in "successful" matings following irradiation with 320r at 10-1/2 days was due, for the most part, to indirect effects of the radiation on the embryos as a result of radiation effects on the maternal organism. By 14-1/2 and 17-1/2 days, the later stages of pregnancy used in this study, gestation had proceeded too far to have been completely interrupted by even a dose of 320r.

Concerning the role of the maternal organism in producing morphological abnormalities in embryos after in utero irradiation, the evidence indicates it is either of no consequence or of very little consequence. Russell (1950) in establishing critical periods for the induction of various abnormalities

in mice reasoned that since the critical periods were very often limited to single days, and since the radiation effects on the dam were probably not distinctly limited to less than a day, any physiological conditions arising in the mother following irradiation would tend to give critical periods that were not sharply delineated. Hicks (1950) detected response to radiation in embryonic tissues as little as two hours after irradiation which would make it appear unlikely that the changes had been mediated through the dam.

In other experiments, for example, Wilson and Karr (1951), individual embryos only were exposed to irradiation with very little maternal tissue being exposed. Embryos exposed in this manner yielded abnormalities in a frequency comparable to that after whole-body irradiation of the mother.

Brent (1957) irradiated rats that were nine days pregnant with 400r but shielded the entire litter with lead so that embryos received less than 0.5r. There were produced none of the grossly observable malformations associated with irradiation of nine day old embryos. Embryos that were so shielded and embryos from unirradiated mothers did not differ significantly in body weights when observed 24 hours before the expected time of delivery.

Grayevsky, et al. (1959) also reported that embryos who were shielded while their mothers were irradiated with 400r and 600r were born with normal rapidity, were of normal weight

at birth, and subsequently had normal descendants. Direct radiation of the embryos, on the other hand, caused a sharp decrease in birth weight and viability. When both dam and embryos were irradiated there was no noticeable intensification of the radiation effect on either body weight or viability of the irradiated embryos.

Additional indirect evidence that morphological abnormalities can be induced by the direct effects of radiation on the developing embryo and need not be a consequence of maternal side effects is found in those cases where the embryos are completely external to the dam so that the maternal intermediary is absent. There are numerous examples in fish, amphibians and birds in which developmental abnormalities have been induced by the direct radiation of embryos.

All of the above examples lend strong support to the view that the effects of in utero irradiation observed in embryos are the results of direct effects upon the embryos. The only serious experimental evidence against this hypothesis is the work reported by Job, et al. (1935). All normal progeny were obtained from pregnant rats whose anterior half was shielded, whereas approximately 40 per cent of the progeny were abnormal in those cases where the dam was exposed to whole-body irradiation. However, none of the subsequent workers who have utilized rats have confirmed these results. It would appear then that most of the developmental

abnormalities are caused by direct effects on the embryo, although the possibility that some of them were dependent on maternal effects cannot be completely excluded.

The influence of the maternal organism on postnatal effects in the embryo has not been adequately explored. Russell (1950) found that mortality after birth was apparently not due to the inability of mothers that had been exposed to whole-body irradiation to care for the progeny. Two females that were given 200r at 10-1/2 days gestation, and one female that was given 300r at 11-1/2 days, and one female given 300r at 12-1/2 days produced litters in which all of the young were dead or moribund. However, when these females were given nonirradiated newborn litters to foster they were able to raise the young to weaning age. No mention is made of the body weights of these foster litters compared to litters raised by nonirradiated mothers.

Rugh (1956a) studied the effects on growth of suckling young from irradiation of lactating mothers. Female mice were irradiated two days after delivery of their litters with single whole-body X-ray doses from 50r to 400r. All litters were standardized to a size of eight young at the start of the experiment. Progeny were subsequently weighed at 3, 5, 8, 10, 12 and 15 days post-parturition. Rugh found that increasing X-irradiation of the nursing mouse caused an increased retardation of the growth of the suckling young. There was a slight

effect observed on the third day post-parturition even after a dose of just 50r, and pronounced effects with doses of 200r or more. It was concluded that there was no evidence of any toxic substance passing through the milk to the young, and that the effect seemed to be one of simple starvation, probably through a general debilitation of the irradiated mother. Newborn mice that had been starved by an irradiated dam recovered rapidly when given to a normal lactating mother at 15 days, and were similar to controls in body weight by 30 days. Rugh reported the results of two separate experiments in his paper, and it is evident that there is considerable variation in the results between the two experiments. For example, in the first experiment progeny raised by mothers that had received 200r weighed only 67 per cent of control weights at 15 days. In the second experiment progeny from mothers given 200r weighed 97 per cent of control weights. The number of mice in each of the sub-classes and the amount of variation in body weight are not given, so that it is not possible to determine which of the differences in body weight are actually significant. Moreover, the body weights of the young before the mothers were irradiated are not given. Random variation of these initial weights could very well influence the subsequent rate of gain. It is difficult, therefore, to make any sort of quantitative generalizations as to the effects of maternal irradiation on growth of the

young. There does appear to be a definite effect on lactation as measured by growth in the progeny but an exact measure of its magnitude cannot be made.

Neither of the above examples provides information on direct effects of *in utero* irradiation of the embryo upon postnatal growth and the indirect effects through changes in the lactating ability of the mother when both of these factors are operative at the same time. The following points in the present experiment provide some indirect evidence which indicates that most of the effects on the postnatal growth of the young are due to the direct effects of irradiation.

1. Irradiation of newborn progeny without the maternal organism receiving any irradiation at all still resulted in severe disturbances in postnatal growth. A dose of 320r given to young at birth produced body weight changes which were similar in magnitude to those obtained after a dose of 320r at 17-1/2 days gestation.
2. After irradiation with 20r or 80r at 6-1/2, 10-1/2, 14-1/2 and 17-1/2 days, postnatal growth was normal, indicating that these dose-embryological age combinations are ineffective in affecting the subsequent lactation of the mother. In addition, a dose of 160r at 6-1/2 days resulted in progeny that were consistently heavier than controls.

3. In the case of progeny irradiated as 10-1/2 day embryos with 80r, body weights significantly lower than controls were not obtained until a month after the young were weaned. This would indicate that the irradiated dam was even capable of overcoming the direct effects of irradiation on the growth of the young, while the latter were nursing.
4. In those other treatments that yielded significantly lower body weights after birth the maximum differences between treated and control progeny were not reached until weaning or later, again indicating that the dam may have been able to compensate somewhat for the effect on the progeny. With these treatments there was little recovery from the maximum relative difference even though a month and a half had elapsed from the time of weaning. If the dam had been solely responsible for slow growth of the progeny through a decreased production of milk, it would seem likely that the young would have made up some of the deficiency when they switched to solid food.

It appears for these reasons that the effects of <u>in utero</u> irradiation upon postnatal growth are due, for the most part, to direct effects of the radiation upon the embryo, and that irradiation of pregnant females has little effect on the lactation of those females. The possibility should not be

excluded that some specific dose-embryological age combination may have an effect on lactation which would be reflected in the postnatal growth of the young. Additional experiments in which females irradiated at different stages of pregnancy are given foster litters to raise would help to better evaluate the direct and indirect effects of radiation on postnatal growth.

The sampling of the entire gestational period by observing effects of X-irradiation given at four different embryological ages, namely 6-1/2, 10-1/2, 14-1/2 and 17-1/2 days, did indicate broad "critical periods" for the induction of changes in postnatal growth. The critical periods are in agreement with those using body weight at birth as a criterion. Russell (1950) found that day 11-1/2 was close to the stage of maximum susceptibility for growth retardation. The birth weights in the present experiment confirmed these results, as of the four embryological stages used, the maximum reduction in birth weights was obtained following irradiation at 10-1/2 days. This stage was followed by 14-1/2 days and then 17-1/2 days and lastly 6-1/2 days. The last two stages actually did not exhibit any growth retardation by birth. The same order of sensitivity was shown in postnatal growth, if allowance is made for the fact that no progeny at all survived irradiation with 160r or 320r at 10-1/2 days. This stage was, however, the only stage in which a dose of 80r had an effect on

postnatal growth. A significantly lower body weight was observed by 60 days of age after irradiation with 80r at 10-1/2 days; whereas 80r at 14-1/2 or 17-1/2 days did not significantly affect body weight. Irradiation of newborn animals produced growth changes which were quite similar to those observed after irradiation at 17-1/2 days. The progeny resulting from irradiation at 6-1/2 days did not exhibit growth retardation at any period in this investigation. The fact that embryos irradiated with 160r at 6-1/2 days always showed a growth acceleration at all periods is probably best explained as being due to smaller litter size. The litter sizes in this group were lower than controls, although not significantly so, and although all weights were adjusted for litter size at birth, no correction was made for the fact that there were different numbers of mice in each litter up to the time of weaning. Number of mice in a litter is known to affect growth rate, and this appears to be the most likely explanation of the growth acceleration in embryos given 160r at 6-1/2 days.

It is important in evaluating the effects of <u>in utero</u> irradiation to consider the time at which observations are made. Thus, in some of the treatments which affected post-natal growth the most, it was not yet apparent by birth or even by 12 days post-parturition that growth would be retarded. For example, embryos irradiated with 320r on 17-1/2 days appear normal at birth and yet are seen later to be some

of the most severely affected progeny. It is obvious that in this case there has not been sufficient time for the damage to be expressed at birth. In addition, the severity of the effect often does not reach its maximum until some time after the first appearance of the effect.

After treatment with 80r at 10-1/2 days, birth weights were significantly lower than controls, but by 12 days post-parturition there did not appear to be any effect on the progeny. If observations had been limited only to that time, it might have been concluded that the progeny had recovered from the initial deleterious effects of irradiation. However, when observed at 60 days of age the progeny were significantly lighter than controls. In this case it is believed that the affected progeny were able to grow normally in the early period of growth while suckling, but that after weaning they were no longer under the direct influence of the mother and effects of the irradiation on the young could be expressed as observed in the lowered body weights. Although postnatal mortality was increased by this treatment, the apparent recovery in body weight was not due to the loss of more stunted progeny. Animals that survived through 60 days had birth weights that were lighter than controls and almost the same as those progeny that died. From these examples it must be stressed that the criteria used to assess irradiation effects should be thoroughly emphasized to avoid misleading

generalizations.

It is of some interest to examine the similarities between these radiation-induced growth changes and growth changes affected by mutant genes, although as Russell (1954) has emphasized, it is unlikely that gene action should exactly parallel the pattern of radiation response. Concerning all the changes that were characteristic of a particular dose-embryological age group, Russell (1950) observed that the changes were more inclusive usually than those changes produced by any single mutant. Even the effects of a highly pleiotropic gene are less numerous than the effects produced by irradiation at a given stage. The value of a comparison of radiation-induced changes and gene-induced changes is that the similarities may indicate common points in the developmental processes that might indicate the time of occurrence of secondary gene effects. The two best known mutants which affect growth in the mouse are pituitary dwarfism (dw) and pygmy (pg). There are some striking dissimilarities between the effects of these genes. Pituitary dwarfism, which was originally described by Snell (1929), causes practical cessation of growth at 14 days. The adult dwarf is but one-fourth to one-third the normal body weight. Some growth retardation is even evident by 7 days. Histologically there is an absence of acidophils by 7 days although the anterior lobe of the pituitary is normal at birth. Other endocrine

glands including the thyroid, thymus, adrenal cortex and gonads are also reduced in size in these dwarfs. However, the primary effect of the gene appears to be on the anterior lobe of the pituitary. When injected with fresh rat pituitaries (Smith and MacDowell, 1930) there was a resumption of growth, and the dwarfs almost attained normal size. All of the other endocrine glands became normal except for the anterior lobe of the pituitary which did not respond to the treatment. Untreated dwarf mice are infertile but the genital system is developed to a certain extent. Smith and MacDowell (1931) showed that dwarf pituitary stimulated growth and engorgement of the uterus and growth of the ovaries in immature female mice indicating that the gonad-stimulating hormones were not directly affected by the dwarf gene.

The pygmy (pg) mutant, first described by King (1950), reduces the six week weight of mice to about one-half that of normal litter mates. The pituitary is small, but it is differentiated into anterior and posterior lobes. In contrast to the condition in the dwarf pituitary there is no hypotrophy of the anterior lobe, and the thyroids and adrenals are apparently normal. The small size of the pygmies is not due to a lack of growth hormone since it was shown that pygmy pituitaries could supply the deficiencies in dwarfs. Reduction in size was already manifest by birth. King concluded that it is possible that the effect of this mutant is to reduce the

responsiveness of tissues of the body to the growth component of the pituitary hormone.

An additional type of dwarfism in the mouse reported by Strong (1948) is apparently different from both pituitary dwarfs and pygmies in that affected animals are small and restless at birth. In competition with normal mice these dwarfs invariably died, but when isolated some survived and one pair produced two litters.

Two examples of growth mutants in the rat which have somewhat different effects than those in mice are the dwarfism reported by Lambert and Sciuchetti (1935) and the dwarf (dw-2) observed by Woolley and Cole (1939). In the former case animals homozygous for this mutant in addition to being dwarfs had thinner coats than normal, cataracts of the lens, and a short lifespan. There was no differentiation of the sexes, and both sexes were sterile. In the case of the dw-2 gene there was no pronounced reduction of growth until the second month of life. Males were always sterile, but females occasionally produced one or two litters.

It is evident from the different examples cited above that the mutant forms of dwarfism have a tremendous variation in their effects on body size, viability and fertility. The experiments of Raynaud and Frilley (1943-1949) are unique in that they attempted to selectively destroy the pituitary of mouse embryos by the use of local X-irradiation of very high

dosage with a narrow beam. The total loss of weight observed in the embryos at term could not be accounted for by just the loss of the directly traversed head tissues. The authors suggest the effects are due to the indirect action of radiation-produced toxins, although these results may also be explained just as well by secondary effects associated with pituitary destruction. In addition to the primary destruction of the pituitary and other head tissues indirect changes were observed in other regions. These changes included reduction of the thyroid and adrenal glands. The adrenal cortex showed both hypodevelopment and hypofunction. Although the pituitary was destroyed sexual development proceeded in a normal or near normal way in both sexes. There was a reduction in the number of germinal cells in the gonads of irradiated fetuses indicating X-irradiation of the pituitary suppresses a special F. S. H. type of gonadotropic function in the fetal hypophysis. The relationships between the various fetal glands are complex, and it is difficult to determine the direct and indirect effects of irradiation on the different glands. The results of these experiments and other experiments involving decapitation of fetuses indicate that the fetal hypophysis contains a growth-stimulating component which normally stimulates growth of the fetus. The sites of action of hormones produced by the fetal adrenal cortex and thyroid are not known, but these hormones may possibly have some effect on prenatal development

and metabolism.

In the present experiment in which the entire embryo was exposed to irradiation it appears likely that in those treatments in which weight changes were observed at birth the impaired growth has been due to effects on various cells throughout the body which produce metabolic derangements interfering with normal assimilation and growth and also to effects on the pituitary which secondarily affected growth of other structures.

In those treatments with which there was a considerable growth retardation some time after parturition, it seems likely that the pituitary has been affected to some degree since the general growth curve is somewhat similar to that produced by pituitary dwarfs. Some of the retardation probably is also due to direct effects of the radiation on other tissues as well as to the secondary effects produced by pituitary disturbances. It will be seen in a subsequent paper that some of the treatments, for example, 320r at 17-1/2 days, produced progeny which were not only stunted, but also had an increased mortality rate throughout life, a higher incidence of cataract formation and decreased fertility. It is not possible from this experiment to determine the contributions to growth retardation due to disturbances in any particular organ or organs in the embryo.

In addition to the effects on growth of <u>in utero</u>

irradiation which were emphasized in this study, certain morphological anomalies visible at term were also observed following irradiation at 10-1/2 days gestation. This aspect of in utero irradiation has been investigated rather extensively recently by other workers including Russell and Rugh in the mouse and Hicks and Wilson in the rat, but a short resume is probably helpful here in understanding some of the underlying causes of the responses to in utero irradiation. The wide variety of malformations induced by radiation in the developing embryo far exceeds effects observed after irradiation of the adult organism. The fact that the embryo as a whole is far more radiosensitive than the adult may be explained by way of the great number of embryonic cells that are undergoing a rapid process of physiological and morphological differentiation. Ionizing radiations are able to disturb this process of differentiation, and the resulting organism may exhibit various types of malformations. The action of radiation probably produces chemical reactions in the cell which inhibit enzymes concerned with the production of nucleic acids and proteins used in differentiating growth. It was noted in this experiment that of the four embryological ages used, the only age in which irradiation produced morphological anomalies visible at birth was 10-1/2 days. The earlier and later stages are not affected, and it is clear the differences in response coincide well with the period of rapid

differentiation of various tissues. The embryological age of 10-1/2 days is near the center of the time when most of the major organ systems are being developed. For example, the overgrowth and reduction of digits observed after irradiation of 10-1/2 days is probably a direct effect of the radiation on the beginning limb bud differentiation. Rugh (1959a) among others, has also shown that the time at which *in utero* irradiation is most likely to produce exencephaly is the time of very active development of the neural tube and head structures. However, the "critical period" for exencephaly may be said to be any time before neural differentiation since exencephaly could be induced by irradiation even within a few hours after fertilization. It is clear then that the embryo may develop congenital malformations following *in utero* irradiation at any stage prior to appearance of the primordia of those organs. Embryos irradiated at 14-1/2 days or 17-1/2 days in the present experiment do not show the anomalies found after irradiation at 10-1/2 days since most of their structures have proceeded too far in their differentiation to be affected in this manner. There are nevertheless some structures that are still undergoing differentiation at these later stages of pregnancy. Hicks (1952) found that the primitive differentiating cells of the nervous system, the neuroblasts,are present late into gestation and even into the first few weeks post-parturition. Rat embryos irradiated

after 14-1/2 days gestation appeared normal externally, but histological examination revealed malformed brains.

The developing embryo also possesses numerous undifferentiated cells which are relatively radioresistant. From these cells the various differentiated cells of the adult organism develop. Although the radiation damaged cells of the embryo are beyond repair or recovery, the embryo does have the ability to organize the remaining undifferentiated cells so that the adult organism then may appear whole and not show any focal deficiencies, but there may seem to be just less of everything, the organism exhibiting certain deletions as a consequence of having had to incorporate certain cells to replace the radiation damaged cells. These deletions may occur as microphthalmia, anencephaly, microcephaly, brachydactyly, stunting, etc. The mouse embryos irradiated at 6-1/2 days with doses up to 160r appeared completely normal by criteria used in this study. They had no gross, external malformations, grew as well or better than controls, and up to the time of this paper are apparently normally fertile. Implantation usually occurs between 4-1/2 and 5-1/2 days in the mouse. The 6-1/2 day embryo is relatively undifferentiated the primitive streak primordium usually appearing around that time. It is possible that the surviving 6-1/2 day embryos were deficient in some manner which was not readily evident. Russell (1954) did find some skeletal and visceral anomalies

following irradiation at this stage. There were also some indications that, at least at higher doses, litter sizes were reduced, indicating severe effects of the irradiation on the developing embryo.

There was usually considerable difference in response to a certain level of irradiation both within litters and between litters. Genotypic differences in response are reflected in the between litters variation in the present study. Allen and MacDowell (1940) have shown that individuals in a litter may differ considerably in their developmental progress at any one particular time, and it is likely that much of these differences within and between litters are due to slight differences in the actual developmental ages of the embryos at the time of irradiation. A very small difference in developmental age during a time of rapid differentiation may result in a large difference in subsequent development.

Although developmental ages of individuals within a litter may be identical or nearly identical, there may still be differences in response to <u>in utero</u> irradiation due to genetic differences in susceptibility to irradiation-induced damage. It was observed in the results on morphological anomalies present at birth that the inbred mice had a higher percentage of malformations (64 per cent) than the hybrid mice (29 per cent). It seems probable that this difference in response does not actually represent genetic differences in

sensitivity to primary radiation damage, but actually represent differences in developmental rates. Hybrid mice, in general, develop more rapidly than do inbreds, and it is possible that the chronological age timed from the vaginal plug represents a different developmental age in each general genotypic class of progeny. The differential responses of the various genotypes used in this experiment to *in utero* irradiation may be explained solely on this basis of different developmental rates. The variation within a litter of genetically homogeneous mice also may be due to different developmental rates.

It is conceivable, however, that the genetic variation in response may be expressed in the secondary effects of the radiation. Thus, within individual cells there may be genuine genetically determined abilities to resist detrimental effects induced by irradiation. Mice of certain genotypes may be able to repair damage and return to normal physiological activity more quickly than mice of other genotypes.

SUMMARY AND CONCLUSIONS

Three genetically differentiated inbred strains of mice and all their possible hybrids, including reciprocals, have been used in this experiment to investigate effects of *in utero* irradiation upon postnatal development. Pregnant females were exposed to single whole-body, 250 pkv X-ray doses on 6-1/2, 10-1/2, 14-1/2 or 17-1/2 days gestation. In addition the study included progeny irradiated on the day of parturition without any irradiation of the maternal organism. The X-ray doses employed were 0r, 20r, 80r, 160r and 320r. Progeny from the irradiated mice were examined at term for morphological anomalies. Postnatal growth was observed from birth to 75 days of age, individuals having been weighed at birth, 12 days, 26 days, 40 days, 60 days and 75 days post-parturition. At 75 days mice that had been irradiated as embryos were mated to untreated mice, and data have been collected on lifetime reproductive performance and total lifespan. The malformations observed at birth and postnatal growth have been reported in this paper, and from these results the following conclusions have been drawn:

1. Morphological anomalies observable at birth were found only after irradiation at 10-1/2 days gestation. Anomalies were observed after doses of 80r or more. Irradiation at earlier (6-1/2 days) or later (14-1/2 and 17-1/2 days) stages yielded

progeny that were morphologically normal at birth.

2. Neonatal mortality was highest following irradiation at 10-1/2 days. The LD_{50} at birth was found to be between 80r and 160r, with a dose of 320r causing all embryos to be stillborn. A small increase in the incidence of neonatal death was observed after a dose of 320r at 14-1/2 days.
3. A differential response both in the induction of malformations and in the incidence of neonatal deaths was found between inbred and hybrid genotypes. After 160r at 10-1/2 days there was among the inbreds 64 per cent abnormal and 100 per cent stillborn progeny compared to 29 per cent abnormal and 64 per cent stillborn among the hybrids.
4. Body weights were adjusted by making use of the pooled regression coefficient of body weight on litter size over all treatments. The embryological age of 10-1/2 days was found to be the most sensitive to growth retardation following irradiation. The remaining stages in order of decreasing sensitivity were 14-1/2 days, 17-1/2 days, newborn and 6-1/2 days.
5. A dose of 80r at 10-1/2 days was sufficient to cause an 11 per cent decrease in birth weights. The maximum reduction was found after a dose of 320r

at 10-1/2 days which caused a 56 per cent decrease in birth weight. Depression in birth weights was also seen after irradiation with 160r or 320r on 14-1/2 days. Embryos irradiated with 160r or 320r at 10-1/2 days or 320r at 14-1/2 days had no survivors or almost no survivors by a few days post-parturition.

6. Of the treated groups surviving the 75 day period, 320r at 17-1/2 days had the greatest effect on growth retardation causing a 25 per cent lowering of body weight at 75 days. Newborn progeny irradiated with 320r had body weights quite similar to progeny from the 320r at 17-1/2 days treatment.
7. In those treatments that produced significantly lowered body weights the maximum effect was usually not found until 40 days post-parturition, and there was little recovery from this maximum by 75 days.
8. Estimates of the components of variation were made within each of the embryological ages for all the growth periods. Genotypic differences in response became maximum 26 to 40 days after birth. Most of the differences were believed due to the early growth advantages of the hybrids over the inbreds since by 75 days there were only small genotypic

effects.

9. Differential responses of the genotypes to levels of irradiation reached a maximum of the total variation at 12 days when an average of almost 50 per cent is due to this effect. The effect has largely disappeared by 75 days.

10. Effects of *in utero* irradiation on postnatal growth were believed to be due mainly to the direct effects of the radiation on the embryos with little of the effect due to secondary effects from irradiation of the mother.

11. An examination of the similarities between the radiation-induced growth changes and growth changes caused by certain mutant genes in rodents was made. It was concluded that the postnatal growth effects observed in this study probably were due in part to disturbances in the pituitary gland and secondary effects associated with it, as well as to direct effect on other organs.

12. Genotypic differences in response to the induction of growth retardation and malformations were thought to be expressed as the result of differences in developmental age of embryos at the time of irradiation and as the result of genetically

determined differences in recovery from disturbed physiological activities.

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APPENDICES

Appendix A

Corrections, in grams, used to adjust body weights; based on the pooled regression coefficient of body weight on litter size at birth over all treatments.

Table 20. Males

Litter size	Birth	Days post-parturition 12	26	40	60	75
2	-.21	-1.4	-4.2	-4.2	-4.2	-1.4
3	-.18	-1.2	-3.6	-3.6	-1.8	-1.2
4	-.15	-1.0	-3.0	-3.0	-1.5	-1.0
5	-.12	- .8	-2.4	-2.4	-1.2	- .8
6	-.09	- .6	-1.8	-1.8	- .9	- .6
7	-.06	- .4	-1.2	-1.2	- .6	- .4
8	-.03	- .2	- .6	- .6	- .3	- .2
9	0	0	0	0	0	0
10	+.03	+ .2	+ .6	+ .6	+ .3	+ .2
11	+.06	+ .4	+1.2	+1.2	+ .6	+ .4
12	+.09	+ .6	+1.8	+1.8	+ .9	+ .6
13	+.12	+ .8	+2.4	+2.4	+1.2	+ .8

Table 21. Females

Litter size	Days post-parturition					
	Birth	12	26	40	60	75
2	-.21	-1.4	-3.5	-2.1	- .7	- .7
3	-.18	-1.2	-3.0	-1.8	- .6	- .6
4	-.15	-1.0	-2.5	-1.5	- .5	- .5
5	-.12	- .8	-2.0	-1.2	- .4	- .4
6	-.09	- .6	-1.5	- .9	- .3	- .3
7	-.06	- .4	-1.0	- .6	- .2	- .2
8	-.03	- .2	- .5	- .3	- .1	- .1
9	0	0	0	0	0	0
10	+.03	+ .2	+ .5	+ .3	+ .1	+ .1
11	+.06	+ .4	+1.0	+ .6	+ .2	+ .2
12	+.09	+ .6	+1.5	+ .9	+ .3	+ .3
13	+.12	+ .8	+2.0	+1.2	+ .4	+ .4

Appendix B

Total number of observations, all genotypes combined.

Table 22. Males

Dose	Embryological age	Days post-parturition: Birth	12	26	40	60	75
0r		45	43	41	41	40	40
20r	6-1/2 days	42	35	34	34	33	32
80r		53	43	40	37	36	35
160r		45	38	38	38	37	37
20r	10-1/2 days	56	50	49	45	42	42
80r		71	56	53	48	47	47
160r		31	0	0	0	0	0
320r		23	0	0	0	0	0
20r	14-1/2 days	54	46	44	42	41	41
80r		52	43	35	34	34	33
160r		75	54	48	47	46	43
320r		26	4	4	4	3	3
20r	17-1/2 days	66	57	51	49	47	47
80r		47	39	37	37	36	35
160r		47	42	39	38	37	37
320r		87	57	40	36	34	34
0r	Newborn	60	46	41	41	41	40
20r		62	38	29	29	29	29
80r		71	45	39	38	38	37
160r		67	31	28	28	27	27
320r		69	37	30	28	27	27

Table 23. Females

Dose	Embryological age	Days post-parturition: Birth	12	26	40	60	75
0r		56	52	48	43	42	42
20r	6-1/2 days	49	32	32	31	29	29
80r		59	49	47	47	47	47
160r		48	31	29	27	26	26
20r	10-1/2 days	48	34	32	21	21	21
80r		76	42	38	35	35	33
160r		39	0	0	0	0	0
320r		28	0	0	0	0	0
20r	14-1/2 days	62	49	46	43	40	39
80r		67	47	42	42	42	41
160r		91	43	39	39	38	38
320r		44	2	2	2	2	1
20r	17-1/2 days	67	53	47	47	46	46
80r		41	33	33	33	33	33
160r		63	49	47	43	43	42
320r		107	49	35	34	27	27
0r	Newborn	61	40	35	34	34	34
20r		67	43	34	33	33	33
80r		83	46	38	37	37	37
160r		66	37	32	21	21	21
320r		108	48	41	38	35	35